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# AN ELECTROPHYSIOLOGICAL STUDY OF THE TROCHANTERAL HAIR PLATE IN THE COCKROACH *PERIPLANETA AMERICANA*

by

© ROBERT KING SUEN WONG

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entittled "An Electrophysiological Study of the Trochanteral Hair Plate in the Cockroach *Periplaneta americana*" submitted by R.K.S. Wong in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physiology.

## ABSTRACT

The anatomical and physiological properties of the trochanteral hair plate in the cockroach metathoracic leg are examined. The hair sensilla of the hair plate can be divided into two groups according to their physical sizes and their relationship with the intersegmental membrane of the coxo-trochanteral joint. A group of approximately thirty long hair sensilla rest on the intersegmental membrane. Another group of short sensilla does not contact the intersegmental membrane. The group of long hair sensilla is displaced by a fold of the intersegmental membrane when the femur is in a relatively flexed position. The physiological properties of the group of long hair sensilla are studied. This group of sensilla is further divided into two types, type I and type II according to their response. Type I sensilla are larger in physical size and produce larger extracellularly recorded impulses than type II. Type I sensilla show only dynamic sensitivity to displacement while type II show sensitivity to both static and dynamic displacements. In the reflex study, it is shown that the activity in the hair plate afferents has an excitatory effect on the femur extensor motoneuron $D_s$ and an inhibitory effect on the femur flexor motoneurons 5 and 6 and common inhibitor neuron 3. Furthermore, afferent activity from the hair plate resets the rhythm of spontaneously occurring rhythmic flexor bursts, indicating that the afferent activity directly influence the flexor burst generator.

Intracellular recordings are made from either the motoneurons,

unidentified nonspiking neurons or afferent terminals within the metathoracic ganglion. The trochanteral hair plate is stimulated either electrically or mechanically. Intracellular recordings from some of the terminal branches of the hair plate showed that high frequency impulses propagating along the afferent fibers may fail to invade the terminal branches causing failure of the postsynaptic response. The failure of impulses in the terminal branches is probably due to a blockage of impulse conduction at the branch points of the afferent fibers within the ganglion. Activity in the hair plate afferents evoked EPSPs and IPSPs in femur extensor and flexor motoneurons respectively. The latency for IPSP is on average 1.8 msec. longer than the latency for the EPSPs. The beginning of the EPSPs follow the peak of the action potentials in the afferent terminals by approximately 0.4 msec. These, and other observations, led to the conclusion that the hair palte afferents monosynaptically excite and disynaptically inhibit the femur extensor and flexor motoneurons respectively.

The effect of removing the trochanteral hair plate in one leg of a freely walking animal is examined. The operated leg oversteps due to exaggerated femur flexion. Correspondingly, recordings from the leg muscles indicate that the flexor burst in the operated leg is more intense and the duration of the burst prolonged. It is concluded that the trochanteral hair plate is normally excited during femur flexion and the afferent activity functions to limit femur flexion and possibly initiate femur extension during a step cycle.

ACKNOWLEDGEMENT

I would like to express my gratitude to Dr. K.G. Pearson for the supervision of this work. The continuing interest expressed by Professor R.B. Stein along with valuable advice is greatly appreciated.

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TABLE OF CONTENTS

## LIST OF FIGURES

# CHAPTER 1

## INTRODUCTION

Insects are excellent animals for the study of neuronal mechanism underlying behavior. They are readily available and inexpensive, their nervous system contains a relatively small number of nerve cell and yet they display a large number of behavior patterns. Within the last decade, a significant amount of studies have been carried out in the insect to investigate the neuronal basis of movements involving rhythmic repetition of muscular contractions such as ventilation, flight, stridulation and walking. An increasing amount of evidence has accumulated showing that the motor patterns underlying these movements are at least partly organized by generators in the central nervous system (ventilation: Miller, 1960; flight: Wilson, 1961; stridulation: Bentley, 1969 a, b; Kutsch & Huber, 1970; walking: Pearson & Iles, 1970; Pearson & Fourtner, 1975). It is also clear that proprioceptors play a significant role in the control of these rhythmic movements. For example, deafferented crickets are unable to successfully oppose the stridulatory apparatus though the rhythmic output continues unchanged (Moss, 1971). In the locust flight system and the cockroach walking system sensory inputs have tonic effects on the frequency of the rhythmic output (Wilson & Gettrup, 1963; Pearson, 1972). At the present time we have very little knowledge on the precise function of feedback from particular proprioceptors in the control of rhythmic movements. The major aim of the work in this thesis is to determine the role of a particular proprioceptor (the trochanteral hair plate) in the control of walking

in the cockroach.

(i) *Types of proprioceptors in the insect leg*

Proprioceptors are sense organs capable of registering deformation (changes in length) and stress (tension, compressions) in the body, which can arise from the animal's own movements or may be due to its weight or to other external mechanical forces (Lissman, 1950). The proprioceptors in the insect leg can be divided into five types, namely the chordotonal organs, the campaniform sensilla, the multipolar stretch receptors, the hair sensilla and hair plate, and the free nerve endings. The histology of some of these proprioceptors has been examined by a number of previous studies (Chordotonal organ: Snodgrass, 1935, Young, 1970; Campaniform sensilla: Moran *et al*, 1971; Multipolar stretch receptor: Guthrie, 1967; free nerve endings: Zawarzin, 1912). In the following sections, the occurrence and the physiological properties of the various proprioceptors are briefly reviewed.

*Chordotonal organ*

The chordotonal organs in the insect leg have been mapped in many groups of insects by Debaisieux (1938). There are usually coxal, femoral tibial and tarso-pretarsal organs. The chordotonal organs are situated below the hypodermis and are derived from the hypodermis. Each organ consists of one to several hundred chordotonal sensilla. An individual sensillum contains one nerve cell and two to three characteristic cells (Snodgrass, 1935; Gray, 1960; Young, 1970). In the mesothoracic leg of the cockroach the coxal and femoral

organs are supplied by a total of about thirty nerve fibers of medium or small diameter (less than 10 μm) (Nijenhuis & Dresden, 1952). Becht (1958) has recorded impulses from the sensory fibers from the coxal organs. These organs are so orientated that they are stretched by extension of the coxo-trochanteral joint. The recorded impulse frequency increased when the organ was stretched. Impulses generating larger extracellularly recorded potentials (larger than 500 μV) adapt quickly and completely. Impulses generating smaller extracellularly recorded potentials (smaller than 500 μV) show incomplete adaptation. The histology and physiological property of the tibio-tarsal chordotonal organ in the cockroach was examined by Young (1970). The sensilla are divided into three groups based on the size and the number of bipolar neurons that are associated with the sensillum. Group 1 sensilla consist of two bipolar neurons, 15 - 20 μm in diameter. Group 2 sensilla consist of two bipolar neurons 8 - 15 μm in diameter and group 3 sensilla consist of a single bipolar neuron about 10 μm in diameter. Electrophysiological records made from the afferent fibers show that organ responds to downward and backward deflection of the tarsus. Group I sensilla show a unidirectional phasic and tonic response to extreme deflection of the tarsus. Group 2 sensilla give a tonic response to the full range of deflection of the tarsus. The characteristics of the response of group 3 sensilla are not described. The femoral chordotonal organ in the metathoracic legs of the locust and grasshopper has been studied by Usherwood *et al* (1968), and Burns (1974) has examined the same organ in the prothoracic and mesothoracic legs of the locust. Recordings from the afferent fibers from the chordotonal organs in the study of Usherwood *et al* revealed two

patterns of activity: a tonic discharge and a phasic rapidly adapting discharge. The frequency of the tonic discharge varies with the femur-tibial angle while the phasic discharge is related to the velocity of tibial displacement. The feedback from the femoral chordotonal organ is found to be important in the control of walking (Usherwood *et al*, 1968).

*Campaniform sensilla*

The campaniform sensilla are located in groups near the joints of the legs in insects. They are small domes of 20 - 30 µm in diameter and in surface view, the sensillum may be circular or oval in shape. There is some variety in the detailed construction of the cuticular part of the sensillum in different insects (Snodgrass, 1935). The sensillum responds to strains in the cuticular skeleton (Demoll, 1917; Pringle, 1938 a, b) and it is innervated by a single large bipolar neuron (Thurm, 1964; Moran *et al*, 1971). Spinola and Chapman (1975) suggested that the excitatory stimulus of the campaniform sensilla of the tibial group 6 in the cockroach is a compression perpendicular to the long axis of the cap of the sensilla and that proprioceptive stimuli (caused by straining the cuticle) also excite the sensillum by indenting the cap of the sensillum. The proprioceptive function of the trochanteral campaniform sensilla has been discussed by Pringle (1940) and Pearson (1972).

*Multipolar stretch receptors*

The histology of the multipolar stretch receptors in the insect leg are generally similar to the receptors described by Finlayson & Lowenstein(1958)in the abdominal segments of various insects. Zawarzin(1912)

located such a cell in the trochanter of *Aeschna* and similar types of cells were noticed in the termite limbs by Richard (1950) and Denis (1958). Guthrie (1967) described three multipolar stretch receptors in the femoro-tibial joint and the femoro-trochanteral joint of the cockroach, The large cell (about 30μm) located near the femoro-tibial joint responded with low frequency, slow adapting trains of impulses to tibial levation. Three multipolar stretch receptors are also located in the femoro-tibial joint of the locust metathoracic leg (Coillet & Boistel, 1968). Increasing the femur-tibial angle resulted in an increasing frequency of discharge from these receptors. The pattern of activity generated is composed of a phasic tonic and a tonic component (Coillet & Boistel, 1969).

*Hair sensilla and hair plates*

In the insect, much of the body surface bears innervated hairs, the histological structure of which was described by Snodgrass (1935). The physiological properties of the hair in the prothoracic segment of the locust was studied by Haskell (1959). Recordings from the afferent nerves of the hairs showed that the sensilla responded to a constant maintained deflection of the hair. The initial frequency of discharge in response to the deflection was high (200 - 300 impulses per second) and bec me adapted to a steady level of 40 - 50 impulses per second which was maintained for a long period of time. The hair sensilla located on the metathoracic tarsal segments of the locust and grasshoppers were studied by Runion & Usherwood (1968). The hair sensilla were described as phasically responsive mechanoreceptors. The discharge frequency of these receptors is proportional to the

velocity of the displacement of the sensory appendages. These sensilla signal the contact of the tarsal segment with the substratum and changes in load during walking (Runion & Usherwood, 1968).

Hair plates are groups of hair sensilla near the coxal-thoracic (inner and outer coxal hair plate), coxal-trochanteral joints of the leg (trochanteral hair plate). The properties of the coxal hair plates and the trochanteral hair plate have been investigated by Pringle (1938 The response of the sensilla of the hair plate was elicited by mechanical stimulation with a needle, the adaptation of the response was described as incomplete and it was commented that the hair plate functioned to register position. In the locust, the hair plates on the smaller sclerites of the neck act together with the prothoracic hair sensilla to provide the animal with the proprioceptive feedback for the proper orientationof the body with the head. The information of the head position relative to the body derived from the hair plates also functions to control grasping movements in the mantis (Mittlestaedt, 1957; Lindauer & Nedel, 1959).

*Free nerve endings*

These are sense cells with branched dendritic processes) ending under the flexible cutivle at the joint membranes (Zawarzin, 1912; Nijenhuis & Dresden, 1952). There has been no attempt to map these nerve endings systematically or to record impulses in their afferent fibers.

(ii) *Proprioceptive control in insect walking*

In the walking insect, it is quite apparent that proprioceptive

tive feedback plays a significant role in shaping the motor output. On indication of this is that there are differences between the motor output recorded from freely walking preparations and de-afferented preparations. In the cockroach, the highest recorded frequency of the reciprocal activity in the levator and depressor motor axons in a de-afferented preparation is significantly lower than the highest frequency of walking (Pearson & Iles, 1970). Similarly, in the milkweed beg, the frequency of reciprocal rhythmic motor bursts recorded from contralateral legs is much lower in immobilized preparations than in intact ones (Hoy & Wilson, 1969). This suggests that leg proprioceptor affects the frequency of the rhythmic motor bursts but is not necessary for the patterning of these rhythmic motor bursts. In the cockroach, proprioceptive leg reflexes induced by forced leg movements followed the input at frequencies higher than those encountered in running (Wilson,1965; Delcomyn, 1971). This observation suggests that the proprioceptive feedback can phasically influence every cycle of the motor output underlying walking movements. A more direct indication of the involvement of proprioceptors in the control of walking is that removal of certain groups of receptor or alteration of sensory input by a change in load can markedly change the motor output associated with the walking movement. In the cockroach, an increase in resistance to leg retraction during normal walking results in an increase in discharge rate of the levator and depressor motoneurons (Pearson, 1972). It was suggested that the trochanteral campaniform sensilla are the receptors responsible for producing the changes in motor output

associated with the change in load. When the femoral chordotonal organ of the metathoracic leg is removed in the locust, the animal can no longer jump (Usherwood *et al*, 1968; Bässler, 1968). Bässler (1968) showed that the locust takes off by a jump only if full flexion is reached between the femur and tibia of the metathoracic leg and that this position of the leg is signaled by input from the femoral chordotonal organs. Reflex studies have shown that in the locust and grasshopper metathoracic leg, the chordotonal organ feedback excites the slow extensor motoneuron innervating the extensor tibiae muscle during imposed flexion of the tibia (Usherwood *et al*, 1968). Following the removal of the chordotonal organ, the speed of walking is lowered and this behavior is correlated with a pronounced decrease in the recorded activity from the slow extensor tibiae muscle. The input from the tarsal receptors in the metathoracic legs of the locust and grasshopper (Runion & Usherwood, 1968) reflexly excites the fast and slow excitatory motoneurons and the inhibitory neurons innervating the extensor tibiae muscle. These receptors are excited when the tarsus contacts the substratum. After the removal of the metathoracic tarsal segment there is a significant fall in the activity of the slow and inhibitory neurons to the metathoracic extensor tibiae muscle when the animal is standing. During walking, recordings from the extensor tibiae muscle reveal that there is a large reduction in the activity of the inhibitory neuron. Wendler (1964) has shown that the hair plates in the proximal part of the legs in the stick insect are part of a feedback system to stabilize the leg position relative to the body.

Removal of the hair plates of the leg caused the operated leg to overstep during walking. However he had not investigated the reflex effect of the hair plates on the leg motoneurons and there is no concise explanation for the overstepping of the leg following the removal of the hair plates during walking.

(iii) *Intracellular recordings from insect neurons*

Intracellular activity in insect neurons was first recorded from cicada motoneurons (Hagiwara & Watanabe, 1956). By using KCl - filled glass microelectrodes, they recorded resting potential and synaptic potentials from motoneurons innervating sound-evoking tymbal muscle. Subsequently, Rowe (1963, 1965) reported successful attempts to record the intracellular neuronal activity in the metathoracic ganglion of the cockroach. The ganglion was first desheathed and intracellular recordings were made from both the cell body region and the neuropil region. Recordings were made in unidentified neurons. The intracellular neuronal activity during rhythmic movements (stridulation) was first recorded by Bentley (1969 a, b) in the cricket. He obtained a preparation of cricket, which after localized brain leision, sang continually for 12 hours. With simultaneous recordings of intracellular activity from the mesothoracic neurons (motoneurons and interneurons) and extracellular activity from motor nerves innervating stridulatory muscles, he was able to correlate the recorded activities and assign them to the closer or opener system. Intracellular activity recorded from opener or closer motoneurons showed rhythmic burst patterns that follow the rhythm of the calling song even in the absence

of any phasic sensory timing. Following Bentley's investigation, a number of studies were conducted to examine the neuronal activity associated with rhythmic movements such flight in locusts (Hoyle & Burrows, 1973 a, b; Burrows, 1973 a, b), rhythmic leg movements and walking in locusts (Hoyle & Burrows, 1973; Burrows & Horridge, 1974) and in cockroaches (Pearson & Fourtner, 1975). Attempts were made in these studies to investigate the central mechanism responsible for producing the motor output underlying these rhythmic movements. In the cockroach walking system, a nonspiking neuron is identified as part of the rhythm generating system (Pearson & Fourtner, 1975). Burrow & Horridge (1974) examined the influence of interneurons and sensory input onto leg motoneurons during imposed and spontaneous leg movements in the locust. One of the general conclusions they reached is that there is a lack of monosynaptic reflex connections in the crustacea and insects. However further investigations in the locust flight system (Burrows, 1975) uncover the monosynaptic connections between the wing stretch receptor neuron and flight motoneurons. Similarly in the cockroach leg, a monosynaptic excitatory connection between the trochanteral hair plate and the extensor motoneuron $D_s$ has been described (Wong & Pearson, 1973) and it seems that the extent of existence of monosynaptic connections in the arthropod cannot yet be assessed until further investigation is conducted in other arthropod systems.

(iv) *Present study*

In the current investigation, experiments are performed

to investigate the properties of the trochanteral hair plate in the cockroach and its reflex effects on motoneurons innervating leg muscles that produce walking movements. Intracellular studies with microelectrodes are carried out to define the synaptic mechanism underlying the reflex pathways from the hair plate to the leg motoneurons. In addition recordings are made in the leg muscles of walking animals both before and after hair plate removal. The changes in the motor output associated with the abnormal walking after hair plate removal are examined. The findings of these investigations provided information on the role played by the trochanteral hair plate in the control of walking.

# CHAPTER 2

## PROPERTIES OF THE TROCHANTERAL HAIR PLATE

### 2.1. INTRODUCTION

At certain points on the integument of the cockroach, most frequently where there is a cuticular overlap, small groups of hairs aggregate to form hair plates. In the legs of the cockroach, there are three groups of hair plates situated in the proximal segments. These three groups of hair plate are the outer and inner coxal hair plates and the trochanteral hair plate. The inner and outer coxal hair plates are situated in the membrane between the coxa and the pleuron, and the trochanteral hair plate is located near the coxo-trochanteral joint. The hair plates are normally excited by a fold of the intersegmental membrane at the joint. The properties of the inner coxal hair plate and the trochanteral hair plate have been investigated by Pringle (1938b). The response of the sensilla of the hair plate was elicited by mechanical stimulation with a needle. The adaptation was described as slow and incomplete and it was commented that the hair plate functioned as "position" sense organs. The involvement of the hair plate receptors in controlling leg movements during walking in the cockroach must be considered probable since removal of the proximal hair plates in the stick insect produced significant changes in the leg movement during walking (Wendler, 1966).

In the present investigation, the properties of the trochanteral hair plate are reinvestigated. The responses of the hair sensilla to step and sinusoidal displacements are studied. The results show that the

hair plate sensilla respond to both static and dynamic displacements.

## 2.2. METHOD

### (i) *The preparation*

Male or female adult cockroaches *Periplaneta americana* were kept in aquarium tanks fitted with wood partitions. The animals were fed with dog chow and water. For experiments to investigate the properties of the hair plate, the animals were lightly anaethestized with carbon dioxide and pinned ventral side up on a cork board. One pin was placed off center near the last abdominal segment and another through the dorsal cuticle just anterior to the prothoracic segment. All studies of the trochanteral hair plate were carried out on the left metathoracic leg of the cockroach. The legs of the animal were extended and fixed with plasticin on the cork board. When the leg was in the extended position, the trochanteral hair plate was exposed.

The trochanteral hair plate is located near the ventral coxo-trochanteral condyle. The axons from the sensilla of the trochanteral hair plate run in nerve 5r5b (notation of Pipa & Cook, 1959). Nerve 5r5b joins nerve 5 of the metathoracic ganglion in the trochanter near the coxo-trochanteral joint (Nijenhius and Dresden, 1955). Detailed description of the anatomy of the trochanteral hair plate will be presented in the results section.

Removal of the cuticle in the ventral surface of the thorax in the metathoracic segment exposed nerve 5. All recordings from the nerves were made with 75 μm silver wire electrodes. Nerve 5 was first cut close to the ganglion. The distal cut end was lifted clear of the haemolymph

and coated with petroleum jelly to prevent drying. The preamplifier used to record from nerve 5 was an Isleworth type A101. These amplifiers were connected to a Tektronix 502A oscilloscope and the output from the cathode followers of this oscilloscope was fed to an Ampex SP300 seven channel tape recorder. All experiments were recorded on magnetic tape. This allowed filming of the records at a latter time.

(ii) *Stimulation of the trochanteral hair plate*

(a) *Mechanical*

The hair sensilla of the trochanteral hair plate were displaced mechanically by a bent insect pin connected to the armature of a vibrator gnerator (Pye-Ling V47). The vibrator was driven by a Hewlett Packard 3300A function generator. Movements of the insect pin were monitored by a length transducer (Hewlett Packard Model 24DCPT-050). The hair sensilla of the hair plate were made to rest on the bent tip of the insect pin. The hair sensilla were pushed by the insect pin in the direction that they would be deflected during femur flexion. The return of the hair sensilla to their resting position was passive.

(b) *Electrical*

A sharp insect pin was used as an electrode to stimulate the hair sensilla of the hair plate. The pin was gently pressed against the base of the hair sensilla. Positive voltage pulses of 0.05 msec. duration, 3-30 volts were applied for stimulation from a Grass SD5 stimulator. The strength of the stimulus was adjusted so that either activation of single hair plate afferents or simultaneous activation of more than one afferents can be evoked from the hair sensilla.

(iii) *Analysis of records*

Some of the experimental data obtained using sinusoidal driving function has been analysed by using a Digital Equipment Corporation LAB-11 computer. All time domain processing of impulses generated by the driving function from single sensillum was accomplished using the PULSE and FNEW-PULSE programs (French, 1970). A cycle histogram is built up when the impulses from the hair plate afferent are generated by a sinusoidal displacement of the hair sensillum. For each histogram 100 cycles were summed and the best fitting sinusoidal curve was calculated (French *et al*, 1972).

## 2.3. RESULTS

(i) *Anatomy of the trochanteral hair plate*

The hair sensilla of the trochanteral hair plate could be distinguished into two groups according to their relationship with the intersegmental membrane of the coxal trochanteral joint (Figure 1). Approximately 30 sensilla, from 30μm to 70μm in length, rested on the intersegmental membrane. Individual sensillum of this group showed a curvature that enabled them to rest along its length on the fold of the intersegmental membrane. Another group of sensilla, located more distal to the joint, did not contact the intersegmental membrane. The length of the sensilla of this group ranged from 5μm to 30μm. Observations under the dissecting microscope revealed that they were not displaced by the intersegmental membrane even during extreme flexion of the femur. All the results in the present study were obtained from the group of longer hairs that contacted with the intersegmental membrane. These

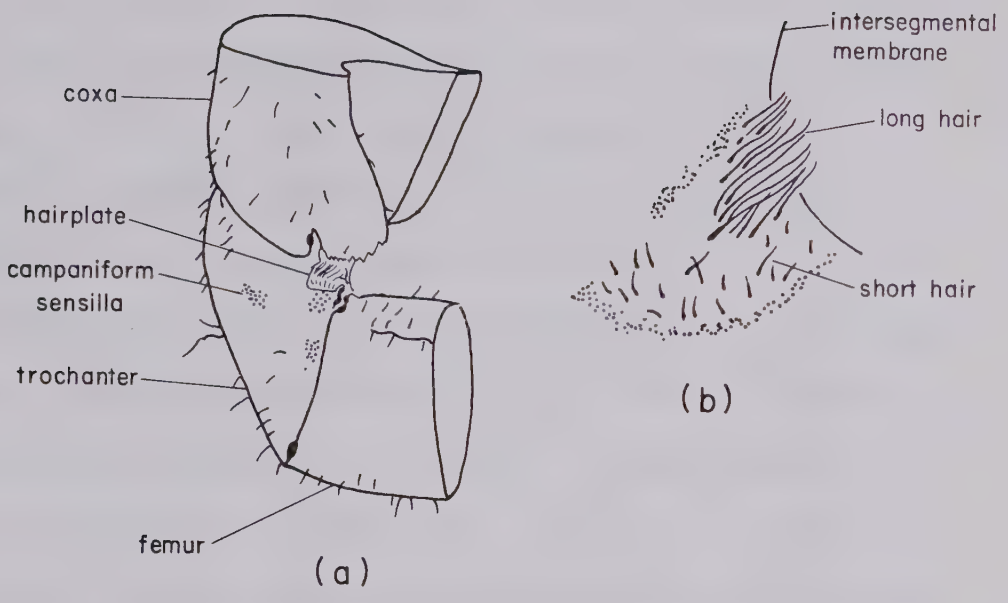


Figure 1. (a) Ventral view of a portion of the left metathoracic leg of the cockroach showing the location of the trochanteral hair plate and three groups of campaniform sensilla in the ventral surface of the trochanter. (b) Enlarged view of the trochanteral hair plate. The long hairs resting on the intersegmental membrane of the coco-trochanteral joint are excited by a fold of the membrane during femur flexion. The samll hairs do not contact the intersegmental membrane and they are not displaced by the membrane even during extreme flexion of the femur.

sensilla were arranged in rows such that a folding of the intersegmental membrane would first displace the most proximal row of sensilla. With further flexion of the femur the increased folding of the membrane would displace the next row of sensilla and so on. Thus the arrangement of the hair sensilla is such that increased numbers of sensilla are displaced when the flexion of the femur is increased.

(ii) *Response to mechanical stimulation*

Individual hair sensilla were separated from the group and stimulated mechanically. Impulses elicited from different hair sensilla recorded by the silver electrodes placed on nerve 5 varied in amplitude. It was observed that impulses with the largest recorded amplitude were always elicited from the longest hair. The group of long hair sensilla normally resting on the intersegmental membrane can be further divided into two types according to their response to mechanical displacement. These two types of sensilla are named type I and type II hair sensilla (see below).

(a) *Step displacement*

When step displacements were applied to the hair sensilla, the onset of the displacement pushed the sensilla away from the intersegmental membrane. This direction of displacement was the same as that along which the sensilla would be displaced during femur flexion. During the release of the displacement, the hair sensilla returned to its initial position.

Type I hair sensilla responded to the onset of the step displacement with an initial phasic discharge followed by a quick and complete adaptation (Figure 2 ). The size of the impulses generated by

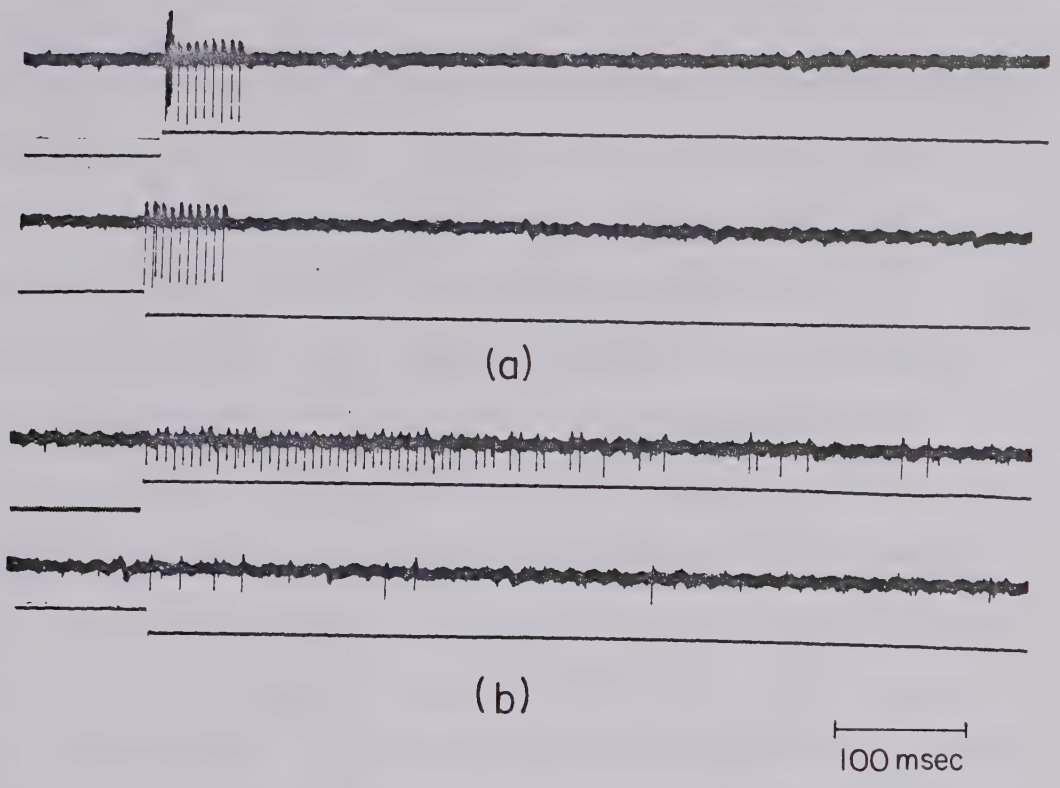


Figure 2. Response of different hair sensillum to step displacement. Upper trace of each record shows the evoked impulses from the hair sensillum recorded from nerve 5. Lower trace signals displacement of the hair. Upward deflection signals onset of displacement when the hair is pushed away from the intersegmental membrane. Downward deflection signals release of displacement when the hair returns to its original resting position by its own elasticity. (a) Type 1 sensilla. Notice the intense initial phasic component evoked by the onset of displacement which is absent during the offset. (b) Type 11 sensilla. The response to the onset of the displacement adapted more slowly than the same response of type 1 sensilla. The response to the release of the displacement is less intense than the response to the onset.

type I hair sensilla recorded by a monopolar electrodes placed on nerve 5 was in the range of 0.3 - 1.0 mv. For type II hair sensilla, the adaptation of the response to the onset of a step displacement was slower and complete for only small displacements (Figure 1 ). For larger displacements type II hair sensilla tonically discharged during a maintained displacement. The magnitude of this static discharge was monotonically related to the degree of displacement. The size of the impulses generated by type II hair sensilla were always smaller than 0.3 mv. Both type I and type II hair sensilla displayed directional sensitivity. For type I sensilla, the initial intense phasic discharge accompanying the onset of the displacement was not evoked by the release For type II sensilla, the onset of the displacement was accompanied by a more intense discharge than the offset of the displacement.

(b) *Sinusoidal displacement*

A large number of hair sensilla, both type I and type II sensilla included, were made to rest on the stimulating insect pin and the sum discharge evoked from the hair sensilla were recorded. Figure 3 shows that the sensilla responded phasically to the imposed displacement. The response showed directional sensitivity, discharges were only elicited from the hair sensilla when they were pushed away from the intersegmental membrane. The return of the hair sensilla to the resting position was not accompanied by any discharge. Within each cycle of displacement, the most intense discharge was always elicited by the maximum velocity portion of the cycle. Responses elicited from type I and type II hair sensilla could be distinguished by the size of the recorded impulse. It can be observed that during low frequencies of displacement (under 10 Hz), type I sensilla responded

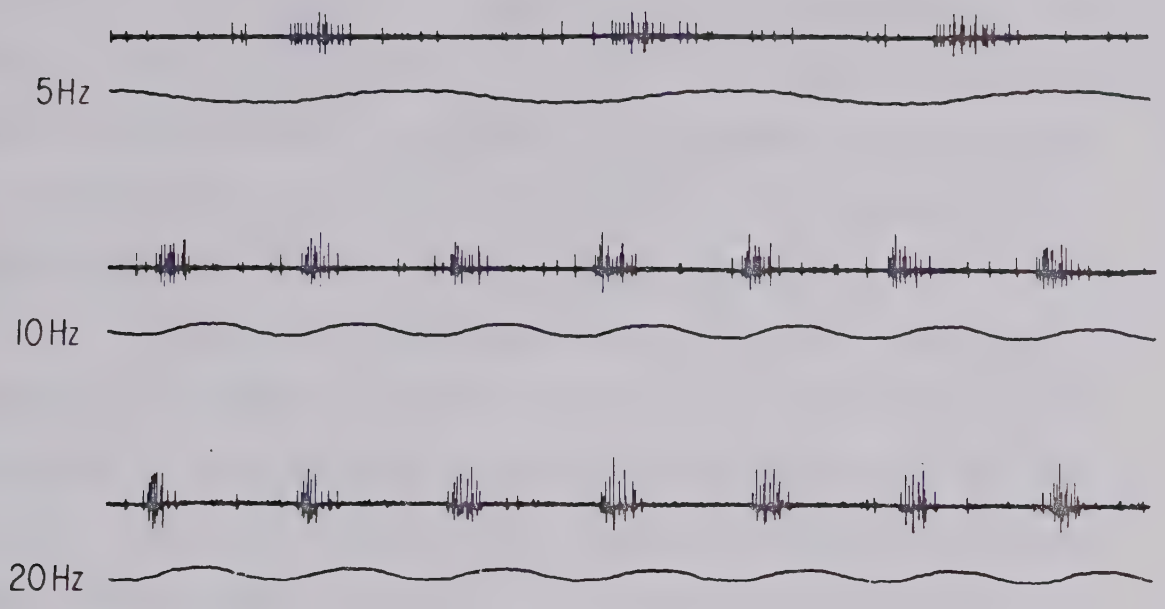


Figure 3. Response of the whole group of long hairs to sinusoidal displacement. The upper trace of each record shows the evoked activity from the hair sensilla recorded by electrodes placed on nerve 5. The lower trace of each record signals the displacement of the hairs. An upward direction shows that the hairs are pushed away from the intersegmental membrane. When the stimulating frequency is at 5 Hz, the impulses with the longest amplitude recorded from nerve 5 are generated by type 1 sensilla. Notice that the maximum response always occurs before the maximum displacement.

to a much smaller portion of the stimulating cycle than type II sensilla. The response of type I sensilla always occurred in the maximum velocity portion of the displacement. As the frequency of displacement increased, the summed discharge frequency of both type I and type II sensilla within each cycle increased. In addition, type I sensilla were recruited to respond earlier in the cycle, although they still responded to a smaller portion of the cycle than type II sensilla.

The response of single sensillum of both type I and type II to sinusoidal displacement is shown in figure 4. The frequency response of both types of sensilla to sinusoidal driving function was analysed. In order to prevent fatiguing of the preparation during threse experiments, the preparation was rested for one minute between periods when the sensillum is stimulated by 100 cycles of sinusoidal displacement of a constant frequency.

During sinusoidal displacement of an individual type I sensillum, it was found that a certain frequency of displacement was required (threshold frequency) before the sensillum responded to the imposed displacement. For various type I sensilla, the threshold frequency ranged from 4 Hz - 12 Hz when the displacement amplitude was 0.02 mm. Senislla generating impulses with the largest recorded amplitudes have the highest threshold frequency. Figure 5 shows the results of an analysis of the response of a type I sensillum to a constant amplitude sinusoidal driving function of 0.02 mm. The amplitude and the phase of the fitted sine curve were both ploted as a function of the displacement frequency. The threshold frequency for this particular type I hair sensillum was 6 Hz. Above the threshold frequency, the

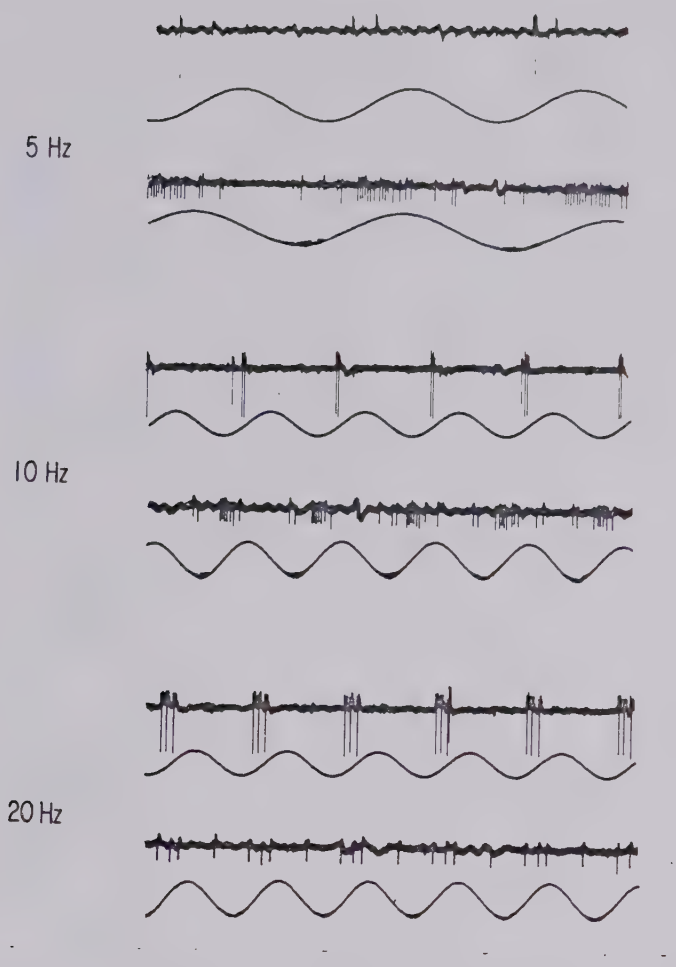


Figure 4. Response of type I and type II sensillum to sinusoidal displacement. The upper traces of each record shows the evoked activity in the hair sensillum afferent recorded from nerve 5. The bottom traces of each record signal the displacement of the hair. An upward deflection shows that the hair is pushed away from the intersegmental membrane. The top records in every frequency of stimulation show the response of type I sensillum and the bottom records show the response of type II sensillum.

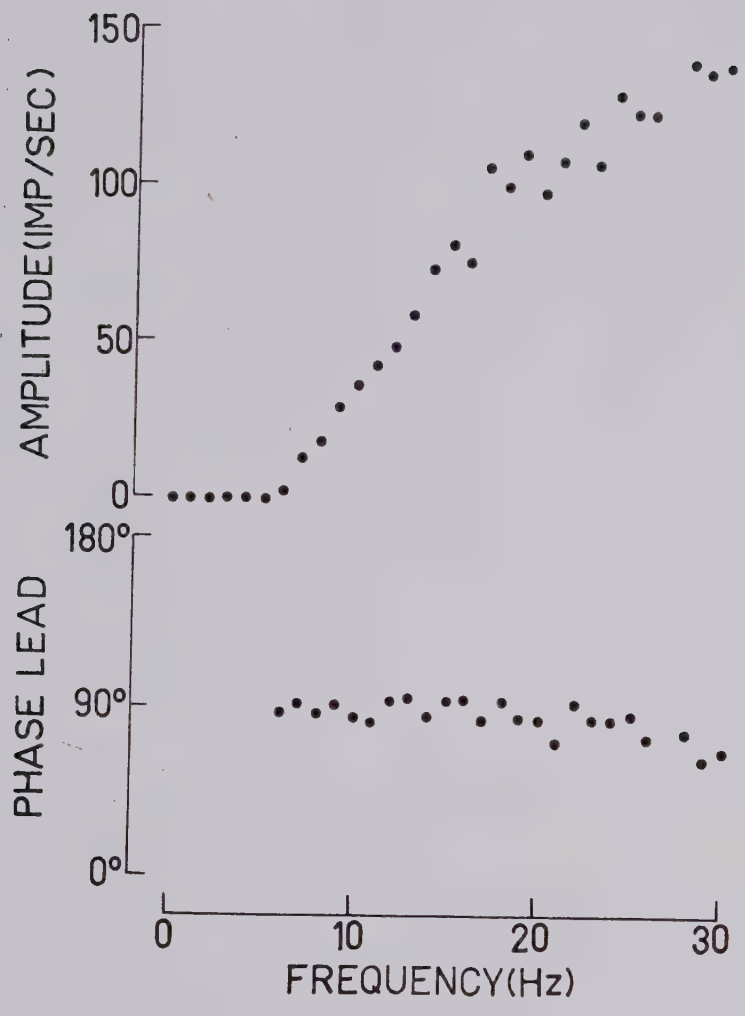


Figure 5. The frequency response function of a Type I sensillum obtained by sinusoidal stimulation. Below 6 Hz no response could be elicited and so the amplitude is zero and the phase is indeterminate. The $90^{o}$ phase lead corresponds to firing on the rising portion of the sinusoid before maximal displacement of the sensillum away from the intersegmental membrane.

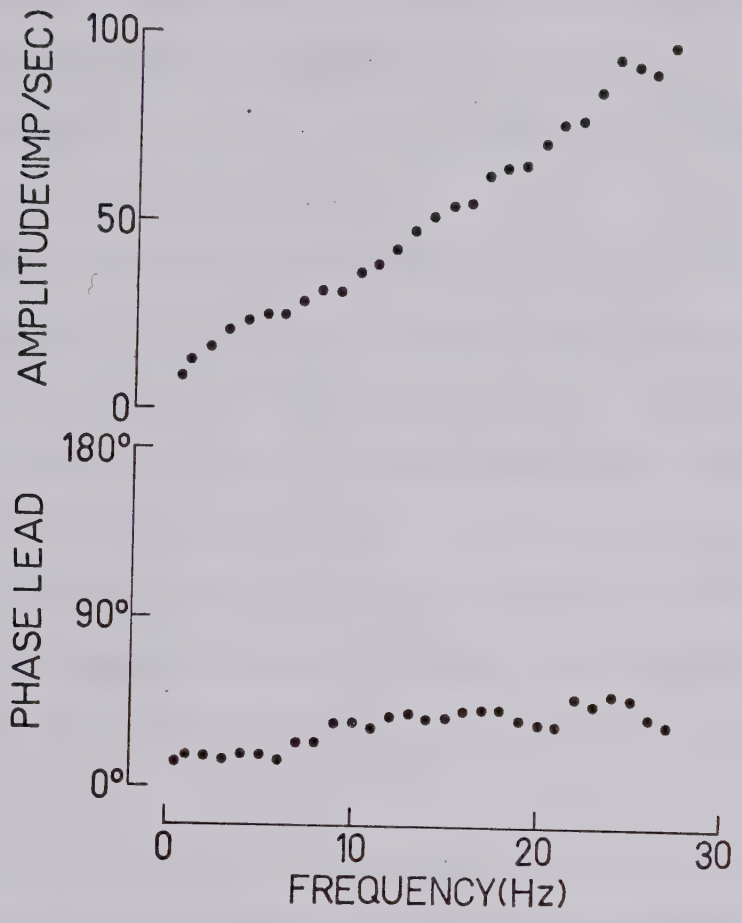


Figure 6. The frequency response function of a Type II sensillum obtained by sinusoidal stimulation. No low frequency threshold could be observed and the phase lead never exceeded 60°.

phase of the sensilla response always occurred at 90° advance of the maximum displacement. The response amplitude of the sensilla is directly proportional to the displacement frequency from 6 Hz to 20 Hz. These results suggest that type I sensilla responded to register the velocity of the imposed displacement.

Figure 6 illustrates the behaviour of a type II sensillum to sinusoidal displacement of 0.02 mm. There are three distinct differences between this and figure 5. (1) The response is never zero valued, even at the lowest frequency (0.5 Hz). (2) The phase of the response is closer to the peak of displacement, never leading it by more than about 60°. (3) The amplitude of response is smaller than the type I by about 30%. These results suggest that the type II sensilla responded to register both the magnitude and the velocity of the imposed displacement.

The lowest frequency of displacement that individual type II sensilla responded to was dependent on the level of displacement. A high level of displacement was required before responses could be elicited when the frequency of displacement was low. (This observation is consistent with the results obtained when step displacements were used to stimulate the type II sensilla. It was observed that a tonic dischange was only elicited when the displacement level was high). The phase of the response however was not affected by the different levels of displacement.

(c) *Punctate stimulation*

In some experiments, a sharp insect pin was used to press

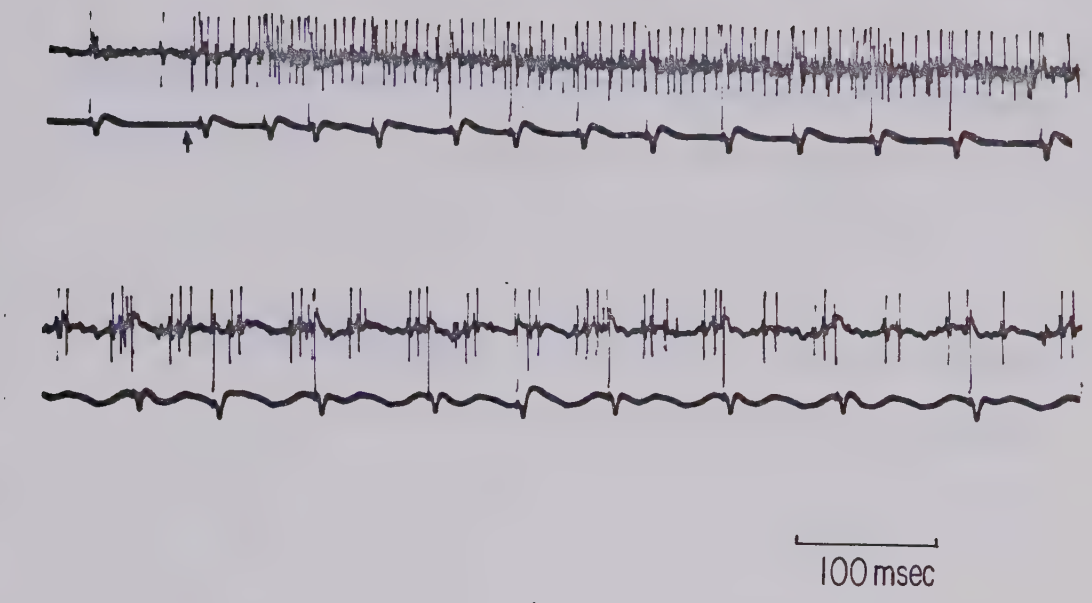


Figure 7. Response of type I sensillum to punctate stimulus and reflex activation of motoneuron $D_s$ by the afferent activity from the sensillum. Top traces - *en passant* nerve 5 record; bottom traces motoneuron $D_s$ activity recorded by electrodes placed in muscle 177D. The arrow in the top record indicate the start of the stimulus. The bottom record is recorded 1.5 sec. after the start of the stimulus. The larger impulse recorded from nerve 5 is the motoneuron $D_s$ impulse identified by a 1:1 correlation with the activity recorded from the muscle. The smaller impulse recorded from nerve 5 is the afferent impulse generated from type I sensillum.

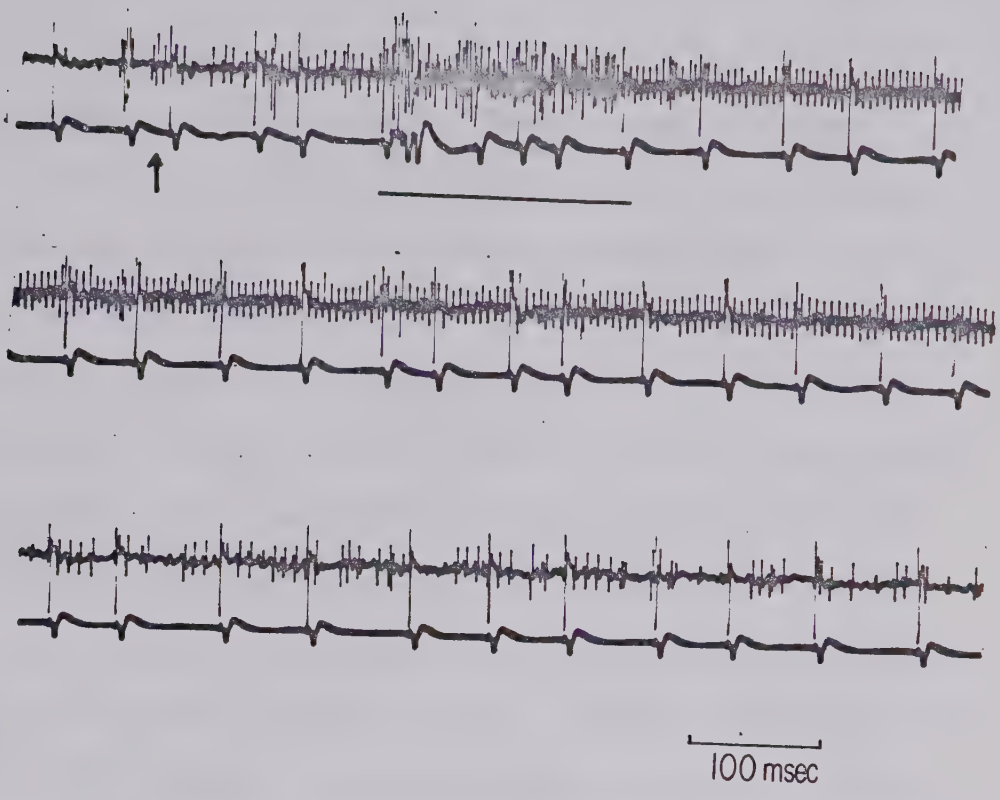


Figure 8. Response of the hair plate sensilla to punctate stimulus and reflex activation of motoneuron $D_s$ by the afferent activity from the sensilla. Top traces - *en passant* nerve 5 record; Bottom traces - motoneuron $D_s$ activity recorded by electrodes placed in muscle 177D. The arrow in the top record shows the start of the punctate stimulus. The intense activity recorded in the top record (indicated by a bar drawn underneath the record) is probably due to a movement of the probe. The larger impulse recorded from nerve 5 is the motoneuron $D_s$ impulse identified by a 1:1 correlation with the activity recorded from the muscle. The smaller impulse recorded from nerve 5 is the impulses generated in the afferent fiber of type II sensillum Middle record is a continuation of the top record. The bottom record is obtained 1.5 sec. after the application of the stimulus.

against the base of the hair sensilla. Usually when sufficient pressure was applied to the base of the hair (by the insect pin), continuous sensory discharge from single sensillum can be elicited. Figures 7, 8 show the response of type I and type II hair sensilla to stimulus applied to the base of specific hairs. A tonic discharge usually resulted from this stimulus. The time course of the response of type I sensilla was not noticeably different from that of type II sensilla. The tonic discharge usually continued for more than one second. It was noticed that a maintained pressure had to be applied to evoke such a response from the sensilla. Removal of the stimulating pin from the base of the hair sensilla always resulted in an abrupt cessation of the response. This result indicates that the response was not caused by an injury of the hair sensilla.

(iii) *Response to electrical stimulation*

Electrical shocks applied to the base of different hair sensilla via a stimulating electrode elicited impulses from the sensillum. The size of the impulses recorded extracellularly by a pair of bipolar electrodes placed on nerve 5 ranged from 0.2 mv to 2.0 mv. Increasing the stimulus strength at any one of the sites of stimulation resulted in the generation of a synchronized response from several afferents. For responses elicited from a single hair sensillum the all-or-none characteristic of the afferent impulse was observed at threshold stimulus strength. For synchronized response elicited from several hair sensilla, decreasing the stimulus strength by a sufficient amount usually resulted in a decrement of the amplitude of the afferent volley. Thus the occurrence of a single unit response and a synchron-

ized response could be readily distinguished.

The conduction velocity for impulses elicited from different hair sensilla was determined by placing two pairs of bipolar electrodes with known distance apart on nerve 5. The time elapsed between the recording of the evoked impulse by one pair of electrodes and the next was noticed. The conduction velocity can then be calculated. The conduction velocity of the afferents from different sensilla ranged from 3.5-5 m/sec. The relationship between the conduction velocity and the amplitude of the extracellularly recorded impulses elicited from the hair sensilla has been systematically investigated. It was found that the relation between the log. amplitude of the impulse and the log. of its velocity of propagation is linear with a slope of 0.4. The amplitude of the extracellularly recorded impulse, then, is proportional to the 0.4th power of the conduction velocity. This relationship between the amplitude of the extracellularly recorded impulse and its conduction velocity is the same as that obtained for the motor axons in the cockroach (Pearson, Stein & Malhotra, 1970).

## 2.4. DISCUSSION

### (i) *Properties of the trochanteral hair plate*

The experimental results suggest that type I hair sensilla responded to register the velocity of the displacement, while type II sensilla responded to register both the magnitude and velocity of the displacement. Both types of sensilla responded transiently to a step displacement. Static discharge of type II sensilla was only evoked when the level of displacement was high. This property of type II sensilla is consistent with the findings some other mechanoreceptors. It has been shown that the evocation of a plateau discharge from a mechanoreceptor often requires a higher stimulus strength than for dynamic response (Catton & PeToe, 1966). The hair plate might therefore have a role in controlling the position of the femur provided that the sensilla are displaced to such an extend that tonic discharge can occur. I have not systematically examined the discharge rate of the trochanteral hair plate at various coxo-trochanteral angles. However it is clear that when the femur is at a slightly extended position, no tonic discharge can be recorded from the hair plate. Hence if the hair plate has a role in position control, it will not be effective when the femur is in an extended position.

Previously it has been suggested that the hair plate functions to register the position of the femur relative to the coxa. This suggestion was based on the finding that the response of the trochanteral hair plate to imposed displacement of the hair sensilla is slowly

adapting (Pringle, 1938b). Our results do not totally support these findings. We conclude that the hair sensilla functions primarily to register the velocity of the imposed displacement (see figure 3). The reason for the discrepancy between the present finding and Pringles observations is not immediately apparent. It should be mentioned that in Pringle's study emphasis was placed on the investigation of the inner coxal hair plate. The results on the investigation of the trochanteral hair plate was not presented, although it was commented that the properties of the trochanteral hair plate were the same as those of the inner coxal hair plate. Furthermore it is possible that in Pringle's study, he had selectively stimulated type II sensilla and had applied a high level of displacement. A more likely explanation for the descrepancy between the present and previous study is that in Pringle's study, the hair sensilla were stimulated by the pressure applied by the stimulating probe to the base of the hairs. The time course of the response of the hair sensilla to these punctuate stimuli obtained in the present experiment is comparable with the records obtained by Pringle.

The present results also suggest that the hair sensilla respond to the direction of the imposed displacement. During sinusoidal displacement, responses are only obtained when the hair sensilla are pushed away from the intersegmental membrane. During step displacement, however, both the onset and offset of the displacement are accompanied by discharges, although the offset is accompanied by a weaker response. These results suggest that the threshold for activation

in both directions of displacement (away and towards the intersegmental membrane) are not the same. A higher velocity of displacement is required before the hair sensilla are activated when the direction of displacement is towards the intersegmental membrane, that is, when the hairs are returning to their resting position. This property of the hair sensilla can be compared with other mechanoreceptors where the threshold for evocation of response by imposed displacements in opposite directions is not the same (Catton, 1970). Since the direction of displacement of the hairs by the intersegmental membrane during flexion of the femur is in the same direction as during the onset of the step displacement, the hair plate is therefore presumably activated only by the flexion of the femur.

The anatomical arrangement of the hair sensilla is such that they are not displaced by the intersegmental membrane until the femur is in a relatively flexed position. Furthermore, the hair sensilla are arranged in rows such that increased flexions of the femur will displace a larger number of hair sensilla. The hair sensilla will therefore only be excited by movements of the femur near the end of the protraction phase of leg movement in a walking animal. Hence the input from the hair plate will provide information about the position of the femur near the end of the protraction phase.

(ii) *Nature of tranducer process and reception sensilla*

The fine structures of the hair sensilla in the locust, tsetse fly and honey bee have bee have been investigated (J.G. Thomas, 1971; Rice, Galun, & Finlayson, 1973; Thurm, 1964). It was discovered that the sensilla functioned through a single primary sense cell -

a large bipolar neuron with a cell body of epidermal origin. The axon of the neuron went to the ganglion and the dendrite was associated with the cuticle. At the tip of the dendrite was the basal body, this basal body gave rise to the cilium. Connected to the distal part of the cilium was the sensory process, which was a membrane bounded bundle of 350-1000 parallel microtubules. The sensory process was connected with the joint membrane in the hair sensilla (the membrane connecting the base of the hair with the surrounding cuticle).

The fine structures of the hair sensillum in the cockroach had not been investigated. However, detailed studies of the structure of campaniform sensillum have been described (Moran, Chapman, and Ellis, 1971). The inner structure of the campaniform sensillum in the cockroach is comparable with that of the hair sensilla in the honey bee. Furthermore it has been shown that the inner structure of the campaniform sensilla and the hair sensilla are the same in the honey bee. Presumably, the structure of the hair sensilla will not be different from the hair sensilla in the honey bee.

It has been suggested that the immediate, effect of a displacement of the hair sensillum is the deformation of the sensory process (Thurm, 1964; Rice, Galum, & Finlayson, 1973). Thurm (1964) suggested that a pinching of the sensory process occurred when the hair was displaced. An isolated compression of the sensory process without displacing the hair achieved by pressing a needle into the base of the hair caused intense discharge. He concluded that a mechanical force which was transverse to the sensory process was the effective stimulus. In the present study, when punctate stimuli applied

to the hair sensilla (pressure on the base of the hair), tonic discharge lasting for more than 1 second can be elicited from both type I and type II sensilla. Presumably, during the application of the punctate stimulus, the deformation of the sensory process is maintained and a tonic discharge is evoked. This indicates that there is only slight adaptation of the spike generating mechanism in the receptor terminal. This adaptation (spike adaptation) is not sufficient to account for the adaptation of the transient response when the hairs are stimulated by displacement. Most probably the adaptation of the sensilla response during displacement of the hairs is caused by the mechanical properties of the sensory process. Presumably the sensory process is only transiently deformed by the onset of a displacement and relaxed during the substained displacement (viscoelastic slip) hence causing adaptation. In the hair sensilla, therefore, it is the mechanical properties of the receptor that is primarily responsible for the adaptation and possibly the difference in the rate of adaptation in the two types of sensilla.

## CHAPTER 3

## REFLEX EFFECT OF THE TROCHANTERAL HAIR PLATE AND THE FUNCTION OF THE HAIRPLATE IN THE CONTROL OF WALKING

### 3.1. INTRODUCTION

The reciprocally patterned motor output in motoneurons producing femur flexion and extension in a cockroach can be produced by the central nervous system in a deafferented preparation (Pearson & Iles, 1970). The legs of the cockroach, however, is equipped with an elaborate system of proprioceptors and the reflexes generated by them during imposed leg movements persist at frequencies of movement higher than those encountered in running (Wilson, 1965; Delcomyn, 1971). A comparison of motor pattern recorded from the deafferented preparation and in an intact animal has led to the proposal that reflexes generated by sense organs do modify the centrally patterned motor output during walking (Pearson, 1972).

Proprioceptors in the legs of the cockroach can be divided into five groups, namely the chordotonal organs, the campaniform sensilla, the multipolar stretch receptors, the free nerve endings and the hair plates (Pringle, 1961; Guthrie, 1967; & Young,1970). The properties of the hair plates in the proximal part of the cockroach leg has been studied previously (Pringle, 1938b) It was proposed that these hair plates are important in the tonic control of leg position. The extend to which these receptors also function in controlling leg movements during walking is unknown but their involvement in walking

must be considered probable since removal of the proximal hair plates in another insect, the stick insect, produces significant changes in leg movement (Wendler, 1966).

In this chapter, the results of a study designed to determine the function of the hair plate receptor in the trochanter of the cockroach metathoracic leg are reported. The reflex effects of the afferents from the trochanteral hair plate on leg motoneurons and the effect of removal of the hair plate on leg movement in a walking animal has been studied. It is concluded from this study that one function of the trochanteral hair is to limit the extent of femur flexion and possibly to promote the initiation of femur extension.

## 3.2. METHOD

All experiments were carried out on the left metathoracic leg of adult cockroach *Periplaneta americana*. The preparation was always kept at room temperature ($20^{\circ}\pm1^{\circ}C$) when experiments were performed. The methods for mechanical and electrical stimulation of the hair plate sensilla have been described in chapter II.

To investigate the reflex effects of the hair plate afferent on leg motoneurons, preparations in which sensory and motor activity could be recorded upon stimulation of the hair plate were developed. To investigate the function of the hair plate in the control of walking, recordings of the muscle activities were obtained both prior to and after the removal of the hair plate. Changes in the motor output before and after hair plate removal were examined. The technique of recording muscular activity in a walking animal has been described previously (Pearson, 1972).

(i) *Anatomy of nerve and muscle*

In previous studies, various motor axons in nerves supplying the metathoracic coxal levator and depressor muscles have been identified and labelled according to the amplitudes of the extracellularly recorded action potentials and discharge patterns (Pearson & Bergman, 1969; Pearson & Iles, 1970). Axons 5 and 6 in nerve 6Br4 innervate the posterior coxal levator muscle 182 (notation of Carbonell, 1947). Activity in these axons produce slow, graded contractions causing flexion movements of the femur. Axon $D_s$ in nerve 5r1 innervates the coxal depressor muscles 177D and 177E. Slow graded contractions of these muscles produced by activity in axon $D_s$ cause extension of the femur. Branches of a common inhibitor neuron reach the periphery via both nerve 6Br4 and 5r1. They are labelled 3 and $D_3$ in each nerve respectively.

(ii) *Extracellular recording of sensory and motor activity*

All recordings from nerves were made with bipolar electrodes made of 75μ silver wire. Nerve 5 of the metathoracic ganglion was lifted clear of the haemolymph and coated with petroleum jelly. This allowed recording of the activity in the afferents from the hair plate and the axon of motoneuron $D_s$ since these axons run in nerve 5. To record the activity in axons 3, 5 and 6 in nerve 6Br4, the animal's left metathoracic leg was rotated to expose the dorsal side of the coxa and nerve 6Br4 was cut just distal to the coxal rim near branch 6Br3, and then retracted medially and placed on the recording electrodes.

In order to monitor the activities in axons 5 and 6 and axon $D_s$ in a walking animal, copper wires of 50μ diameter, insulated

to the tip, were inserted in specific regions of muscles 182C and 177D respectively (Pearson, 1972). The excitatory junctional potentials elicited by axons 5 and 6 and axon $D_s$ could then be recorded.

## 3.3. RESULTS

### (i) *Reflex effects of the hair plate on motoneuron $D_s$*

#### (a) *Response to mechanical stimulation*

The onset of a step displacement of the hair sensilla of the hair plate transiently increased the spontaneous activity of motoneuron $D_s$ (figure 9). This response in motoneuron $D_s$ adapted quickly although the displacement of the hairs was maintained. The release of the displacement was accompanied by a much weaker excitation of motoneuron $D_s$. It has been shown that the hair sensilla responded with a more intense discharge during the onset of the step displacement than during the release(figure 2). This property of the hair sensilla can account for the much stronger reflex response of motoneuron $D_s$ during the onset of the step displacement of the hair sensilla.

Figure 10 shows the reflex response of motoneuron $D_s$ during sinusoidal displacement of the hair sensilla of the hair plate. Motoneuron $D_s$ was reflexly activated only when the hairs were pushed away from the intersegmental membrane. The return of the hair sensilla to the resting position did not activate any motoneuronal response. During low frequencies of displacement, the reflex response of motoneuron $D_s$ superimposed on its spontaneous activity. Above 15Hz, the spontaneous activity of the motoneuron was suppressed and the reflex response of motoneuron $D_s$ could be elicited in a 1:1 manner up to 90 Hz. However, at this high frequency of hair sensilla displacement, the reflex

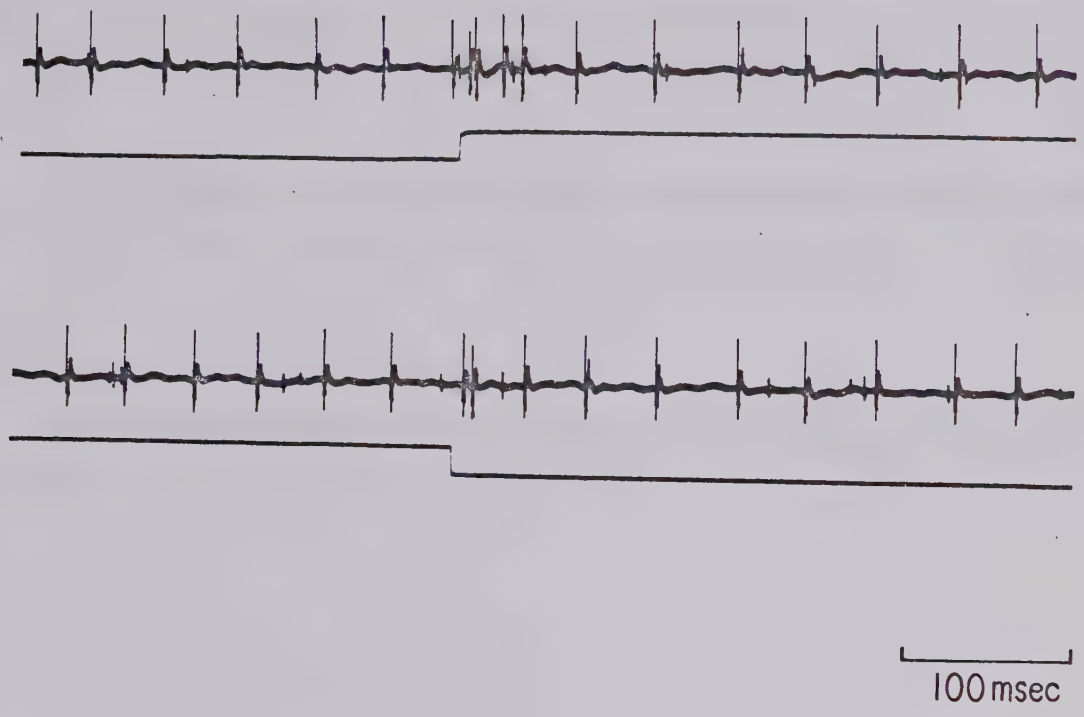


Figure 9. Response of motoneuron $D_s$ to step displacement of the hair sensilla. Upper trace motoneuron $D_s$ activity recorded by electrodes placed in muscle 177D. Motoneuron $D_s$ is spontaneously active. Lower trace signals the displacement of the group of long hair sensilla. Upward deflection signals onset of displacement and downward deflection signals release of the displacement.

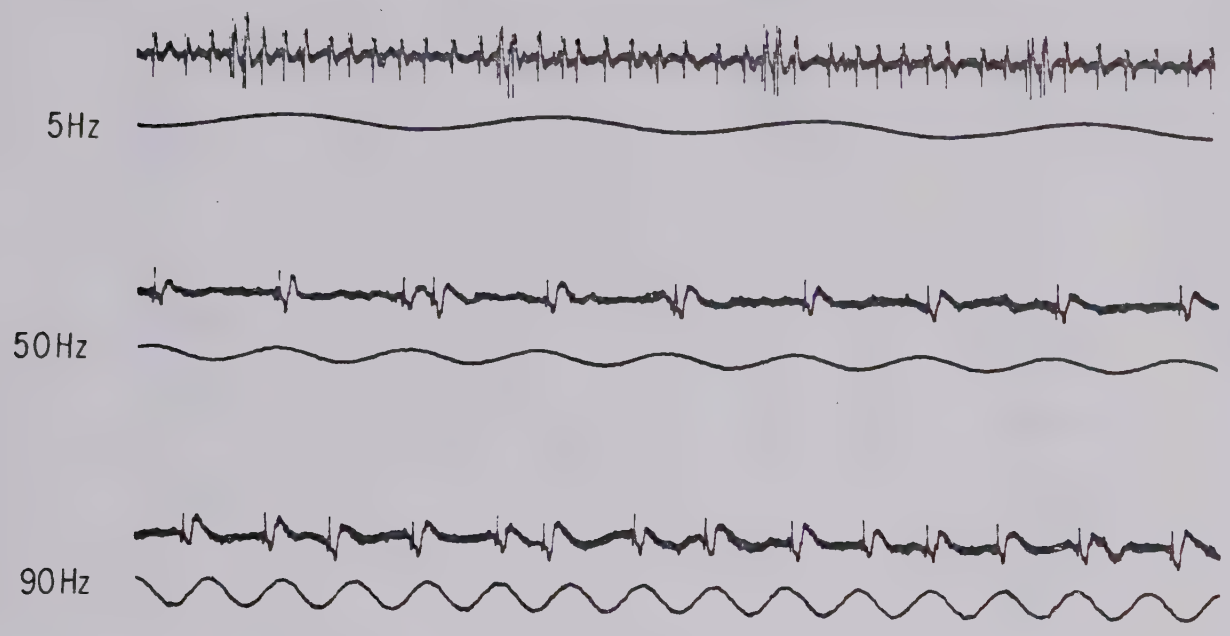


Figure 10. Response of motoneuron $D_s$ to sinusoidal displacement of the hair sensilla. Upper trace motoneuron $D_s$ activity recorded by electrodes placed in muscle 177D. Notice the shape of the recorded potential at 50 Hz and 90 Hz of hair displacement. The initial spike is caused by the impulse propagating in axon $D_s$ and the longer diphasic (negative-positive) potential is caused by the muscle potential evoked in muscle 177D by the nerve impulse (positive direction upwards). Lower trace of each record signals the displacement of the hairs. Upward direction signals that the hairs are pushed away from the intersegmental membrane. Note the 1:1 following of the motor response when the displacement frequency is as high as 90 Hz.

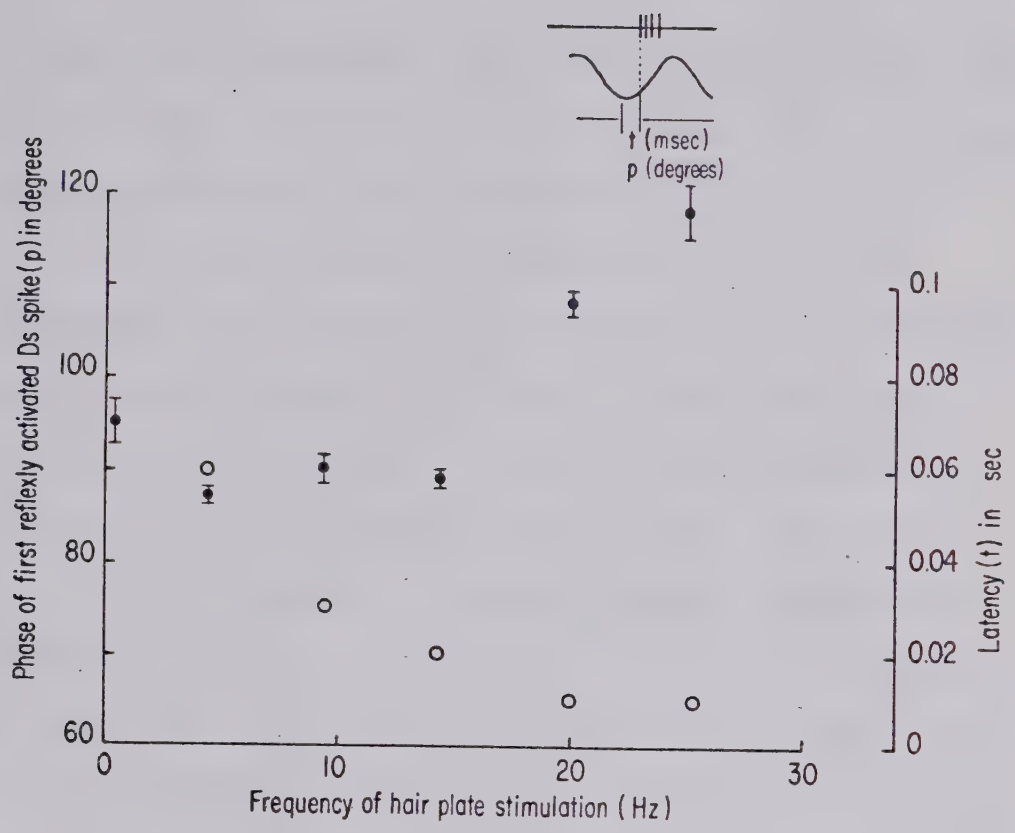


Figure 11. A plot of the phase and the latency of the first reflexly activated motoneuron $D_s$ spike in the stimulus cycle applied to the hairs of the hair plate. The inset shows the points on the record from which the phase and latency are measured. The top trace of the inset shows a drawing of the reflexly activated motoneuron $D_s$ spikes recorded from the muscle; the bottom trace signals the displacement of the hairs. Filled circles are a plot of the phase and open circles are a plot of the latency. The bars above and below the circles indicate standard deviation.

response adapted after the initial one or two seconds. At frequencies of displacement lower than 50 Hz, the reflex response can be elicited for tens of seconds without showing adaptation.

The position of the first reflexly activated $D_s$ spike in a stimulus cycle at different frequencies of hair plate stimulation was measured. Figure 11 shows a plot of the results. Below 15 Hz, the first $D_s$ response was always activated at 90 degrees before the maximum displacement of the stimulus cycle occurred. Above 15 Hz, the phase advance decreased and at 25 Hz of stimulus frequency, the phase advance of the first $D_s$ reflex response decreased to 60 degrees. The time elasped since the start of the stimulus cycle (when the hair sensilla were in the resting position) to the occurrence of the first $D_s$ response at different frequencies of stimulus was measured. This gave the latency of the first $D_s$ response. Figure 11 shows that when the stimulus frequency was below 20 Hz, the latency decreased with an increase in the frequency of stimulus. At 20 Hz, the value of the latency was 10 msec. and further increase of the stimulus frequency did not caused any further reduction in the latency.

(b) *Response to electrical stimulation*

In order to study the latency of the reflex response of motoneuron $D_s$, electrical stimulation was used to excite the afferents from the hair plate. Simultaneous activation of a number of afferents excited motoneuron $D_s$ after a short latency. Figure 12 shows a record of the synchronized sensory volley and the motoneuron $D_s$ impulse response recorded by a pair of bipolar electrodes placed on nerve 5.

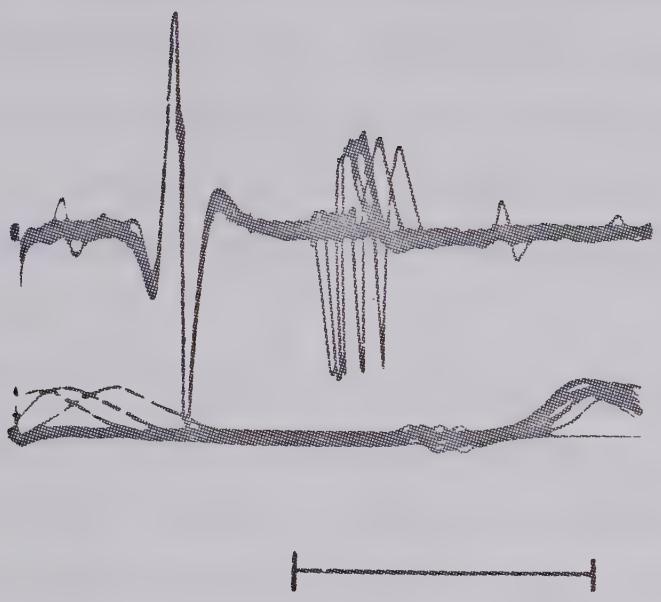

Figure 12. Records show the electrically evoked synchronized afferent volley from the hair plate sensilla and the response of motoneuron $D_s$. Notice that the sensory volley and the motoneuron impulse are opposite in the order of their positive and negative phases. Lower trace is the recording in muscle 177D. Eacch efferent impulse always generates a muscle potential in muscle 177D, showing that the efferent impulse is evoked in motoneuron $D_s$.

It can be observed from the record that the sensory volley and the motor impulse are opposite in the order of their positive and negative phases. This feature of the recorded extracellular activity enable one to differentiate between sensory volleys and motoneuron impulses travelling in the opposite direction in a mixed nerve (Stein & Pearsson, 1971). In addition, the motoneuron $D_s$ impulse recorded on nerve 5 was identified by a 1:1 correspondence with the junctional potentials recorded from muscle 177D.

Impulses elicited from a single sensillum of the hair plate produced an excitatory effect on the activity of motoneuron $D_s$. This effect was seen for both type I and type II sensilla (recognized by size of extracellularly recorded impulse). Thus both types of sensilla established excitatory connection with motoneuron $D_s$. Figure 13a shows the distribution of latencies of the impulse in motoneuron $D_s$ following an impulse elicited in a single hair sensillum. There was considerable variation in the latency of the response. The variability in latency was reduced when the stimulus strength was increased to elicit a synchronized volley from several hair sensilla (figure 13b). The minimum latency of the motoneuron response, however, was not affected by the changes in strength of the afferent input. Following the synchronized sensory volley, the motoneuronal response was evoked after a short delay. Figure 13b shows that the occurrence of the motoneuronal response tends to peak with a delay of 2.2 msec. following the sensory volley. This value of delay was taken as the minimum reflex delay measured at the point of recording on nerve 5. Any motoneuron impulse occuring sooner than 2.2 msec. following the sensory volley were most probably generated spontaneously and were not considered to

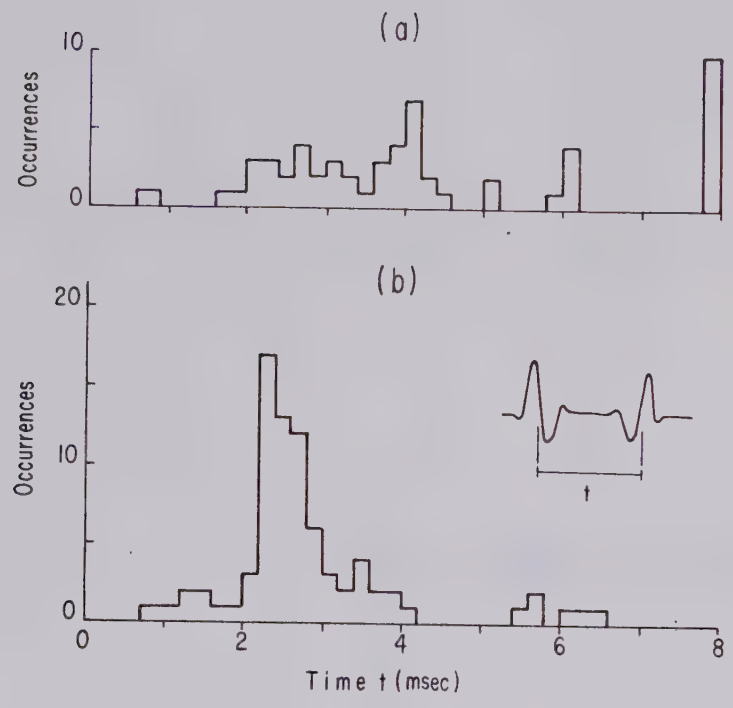


Figure 13. Histogram of the latency of the spike evoked in axon $D_s$ by afferent activity of the hair plate. Points on the recorded afferent volley and efferent impulse taken for the measurement of latency are shown in the inset. (a) Latency of motoneuron $D_s$ response following the activation of a single hair sensillum. The number of responses occurring later than 8 msec. following the afferent impulse is shown in the last column. (b) Latency of motoneuron $D_s$ response following simultaneous activation of a number of hair sensilla. Motoneuron $D_s$ is spontaneously active; impulses occurring sooner than 2.1 msec. following the afferent input are most probably generated spontaneously and are not considered to be reflex responses (see text).

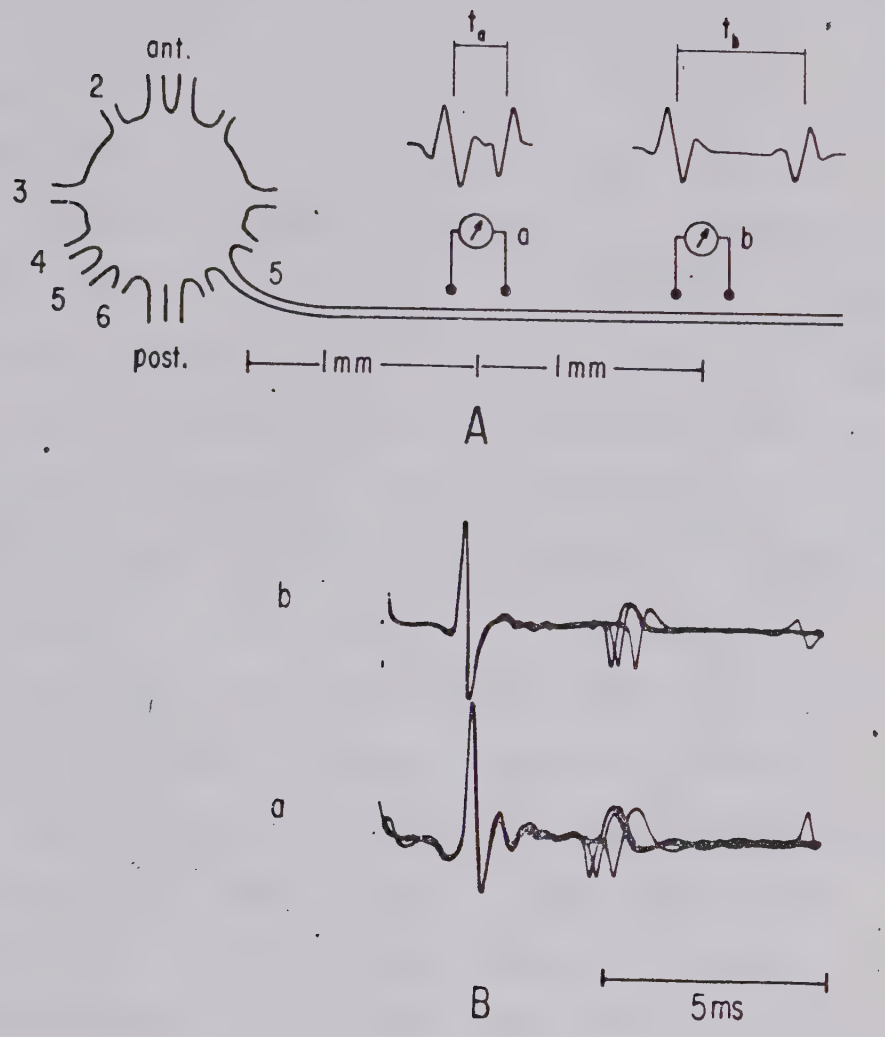


Figure 14. (a) Diagram showing the arrangement of electrodes for the measurement of central latency. The reflex latencies recorded by electrode pair a and pair b are $t_a$ and $t_b$ respectively. $t_b - t_a$ = time for afferent and efferent activity to propagate 1 mm. Therefore central latency = $t_a - (t_b - t_a)$ msec. (b) Upper trace: record of afferent and efferent activity recorded by electrode pair b. Lower trace: afferent and efferent activity recorded by electrode pair a.

be reflex responses.

To measure the minimum central latency of the reflex, two pairs of bipolar electrodes were placed on nerve 5. The proximal pair of electrodes were placed at a distance of 1 mm. from the root of nerve 5, and the distal pair of electrodes were placed at a distance of 1 mm. from the proximal pair (figure 14a). A synchronized sensory volley was then evoked to activate motoneuron $D_s$. Figure14b shows the sensory volley and the motor impulse recorded with the electrode arrangement illustrated in figure 14a. The difference in the reflex latencies recorded at the distal and proximal pairs of electrodes gives the sum of the time required for sensory volley and motoneuron $D_s$ spike to propagate 1 mm. By subtracting this sum from the reflex latency recorded by the proximal pair of electrodes the central delay can be obtained (see legend of figure 14 ). From five preparations, the minimum central delay was calculated to be 1.3 msec. Thus with this electrode arrangement, the central delay can be calculated without having first to compute the propagation velocity of the sensory volley and motor spike.

(ii) *Reflex effect of the trochanteral hair plate on flexor motoneurons*

Onset of the step displacement of the hair sensilla produced a transient inhibition of the tonic activity of flexor motor axons 5,6 and the common inhibitor axon 3 (figure 15). It was observed that the duration of inhibition of the neurons was directly related to their size. Thus the activity of axon 3, the axon of the smallest neuron, was inhibited for the shortest period of time. The duration of inhibition for axon 6, the largest axon that was active during the

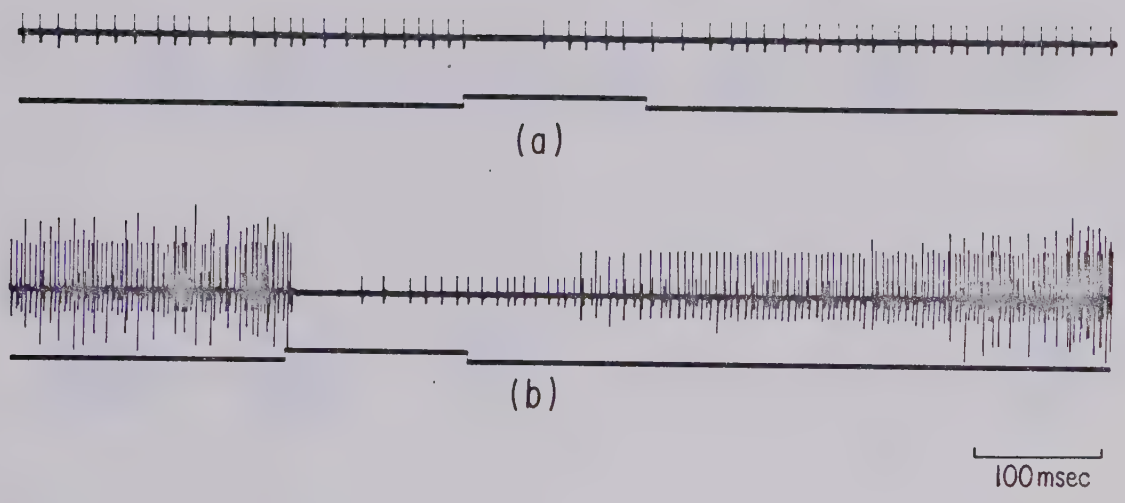


Figure 15. Record showing inhibition of tonic activity of flexor motoneurons 5 and 6 and common inhibitor neuron 3 by a displacement of the hairs of the hair plate. Top trace shows the acticity of axons 3, 5 and 6 recorded from nerve 6Br4. The impulse with smallest recorded amplitude is generated in axon 3. The impulse with the largest recorded amplitude is generated in axon 6. The lower trace of the record signals the displacement of the hairs. An upward deflection indicates that the hairs are pushed away from the intersegmental membrane and a downward deflection indicates the return of the hairs to the resting position.

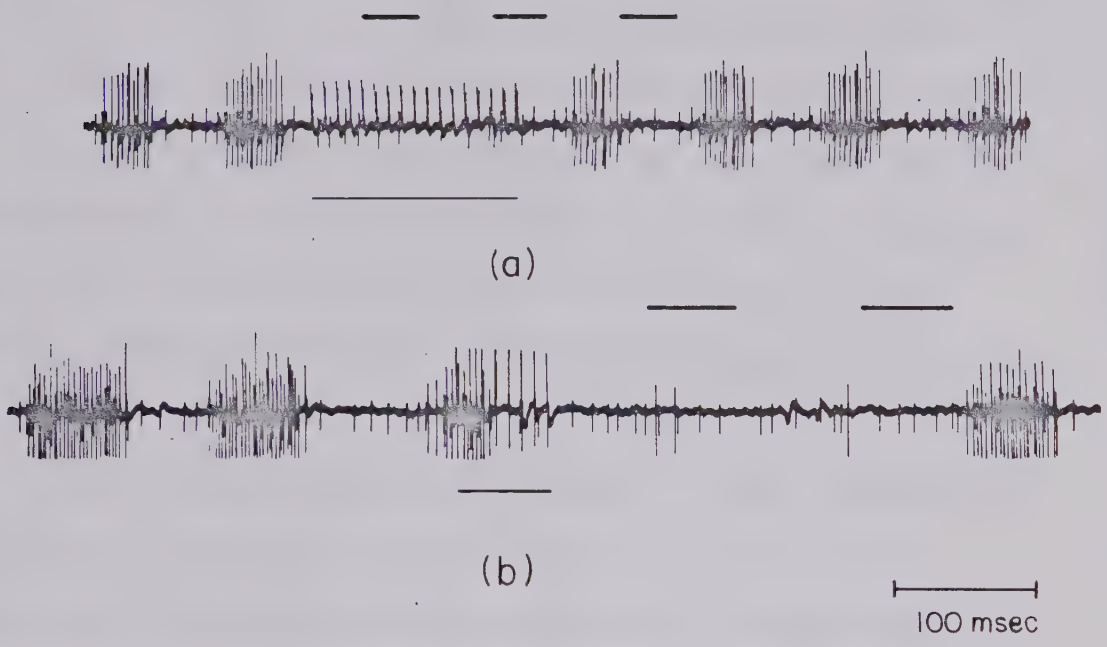


Figure 16. Records of inhibition of rhythmic flexor burst by repetitive electrical stimulation of the hair sensilla of the hair plate. Activity of axons 3, 5 and 6 is recorded by electrodes placed under nerve 6Br4. The application of electrical shocks is indicated by the line drawn under the nerve record. The stimulus artifact generated by the shock can be observed. The bars above the nerve record indicate where the flexor bursts would have occurred had the stimulus not been applied. (a) The electrical shocks were applied between the flexor bursts. (b) The electrical shocks were applied during the flexor burst.

displacement of the hair sensilla, was the longest. Single afferents, excited by electrical stimulation of the hair sensilla of the hair plate did not produce any observable inhibition of the activity of axons 3, 5 and 6. Synchronized volleys from several hair sensilla had to be evoked before the activity of axons 3,5 and 6 could be inhibited. A rough estimate of the latency of inhibition was obtained by measuring the time elapsed from the onset of the step displacement to the suppression of the tonic activity. The latency obtained was less than 10 msec.

Rhythmic bursting activity of axons 3, 5 and 6 generates the flexion movement of the femur during walking. Previous study has shown that these rhythmic bursts can be generated in de-afferented preparations (Pearson & Iles, 1970). We have found that stimulation of the hair plate with high frequency shocks (100 Hz) influenced the timing of these rhythmic bursts. Preparations with cut thoracic connectives were used for this study since rhythmic bursts of axons 3, 5 and 6 could be elicited more easily. Figure 16a shows that when the hair sensilla of the hair plate were stimulated between the flexor bursts, there was a suppression of the burst activity. Burst activity then resumed after a delay following the cessation of the stimulus to the hair sensilla. The occurrence of the first burst following the stimulus was not usually at the instant when a burst of activity would have been expected had the hair sensilla not been stimulated. In other words, the rhythm of the flexor burst was reset by the afferent activities from the hair plate. In other instances when the hair sensilla were stimulated during a flexor burst, there was a shortening of the flexor burst in addition to the inhibition of the next flexor

burst (figure 16b ).

(iii) *Effect of removal of the hair plate on the activity of the flexor motoneurons 5, 6 and the extensor motoneuron* $D_s$

The hair plate can be easily removed by a pair of forceps with minimum damage to the surrounding cuticle. Subsequent to removal, flexion of the operated leg was exaggerated during walking. This overstepping effect caused the operated metathoracic leg to collide with the ipsilateral mesothoracic leg during flexion. The same effect on leg movement (overstepping) has been described by Wendler (1966) when the coxal and trochanteral hair plates were removed in the stick insect.

Recording of muscular activities in an operated animal enabled me to examine the changes in motor output which were associated with the abnormal leg movements following hair plate removal (figure 17 ). An analysis of results in two animals revealed that (1) the duration of the flexor burst in the operated leg was prolonged compared to the one in the ipsilateral normal leg (figure 18 ), (2) the intensity of the flexor burst was increased (figure 18), and (3) the initiation of the extensor burst following the end of the flexor burst in a step cycle in the operated leg was not delayed.

## 3.4. DISCUSSION

The function of the trochanteral hair plate in the control of walking has not previously been examined. In chapter 2, it has been shown that the trochanteral hair plate is activated by the phasic flexion movements of the femur. The results in this chapter show that the hair plate afferents have a strong reflex influence on the motoneurons

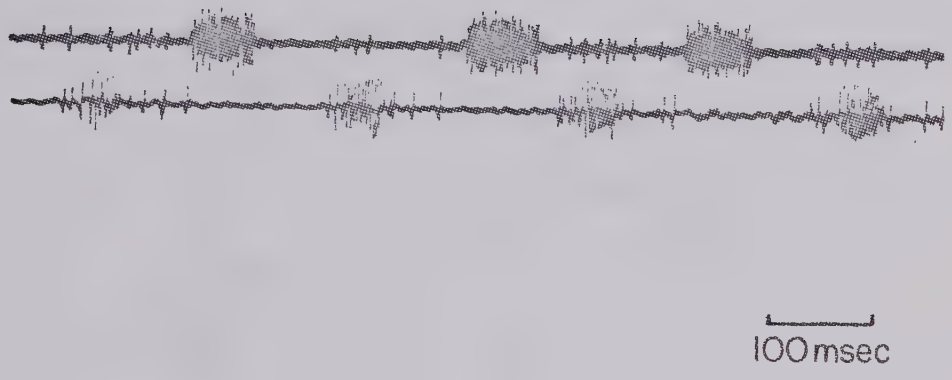


Figure 17. Burst activity recorded from coxal levator muscles of the metathoracic legs of a freely walking cockroach. The top trace is recorded from the muscles in the leg where the hair plate is removed. The bottom trace is recorded from muscles of the normal contralateral leg.

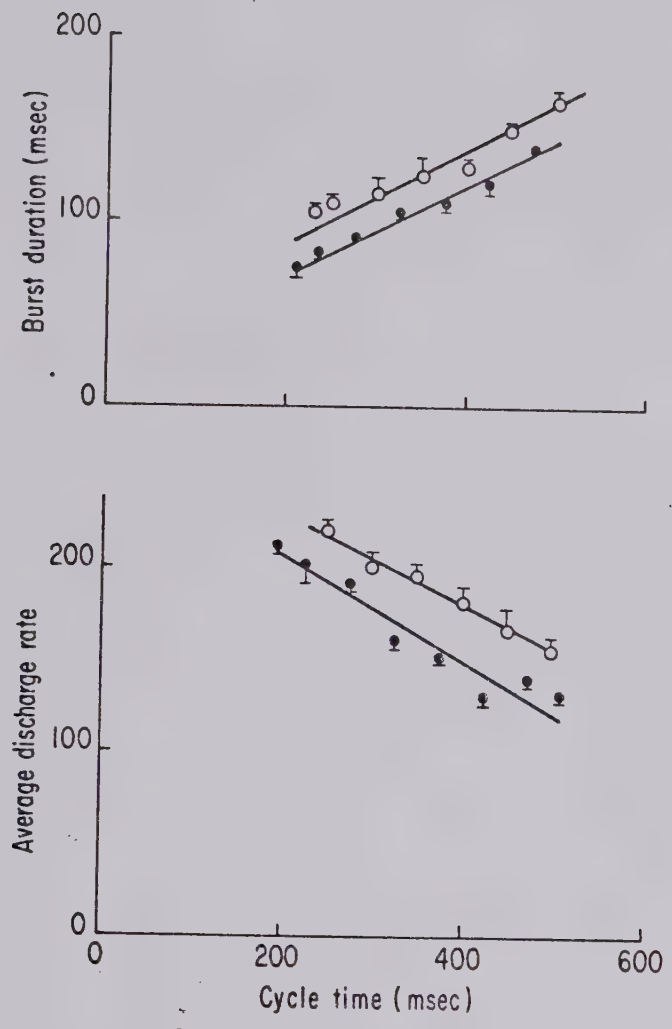


Figure 18. Graphs showing the effect of removal of trochanteral hair plate in one leg on the burst duration and average discharge rate of the flexor burst in the operated leg during walking. Open circles are a plot of the record obtained after hair plate removal. Closed circles are a plot of records obtained before hair plate removal. The bars above and below the circles indicate the standard deviation. (a) Graph of burst duration versus cycle time. (b) Graph of average discharge rate versus cycle time.

that produced femur extension and flexion movements. Furthermore removal of the hair plate resulted in overstepping movements of femur in the operated leg during walking. These results indicate that the trochanteral hair plate has a role in the control of walking.

(i) *Reflex effect of the trochanteral hair plate afferents onto the femur extensor and flexor motoneurons*

The afferent activity of the hair plate reflexly excites the femur extensor motoneuron $D_s$ and inhibits the flexor motoneurons 5 and 6. The excitatory connection with motoneuron $D_s$ can follow high frequencies of afferent input, and the minimum central latency of the reflex is short (1.3 msec.). The time for this latency includes (1) sensory conduction time within the ganglion, (2) synaptic delay, (3) time for the rise of EPSP to threshold, and (4) motor conduction time within the ganglion. Assuming that the synaptic site was 200μ from the root of nerve 5 and the average motor and sensory conduction time was 4 m/sec., then (1) plus (4) amount to 0.1 msec. Assuming that the rise of EPSP to threshold requires 0.5 msec., an estimate of the synaptic delay will be 0.7 msec. This short synaptic delay and the high 1:1 following frequency of the reflex motor response suggest that the excitatory connection is monosynaptic. In the next chapter it will be confirmed that this is so.

The first reflexly activated $D_s$ spike was elicited at a constant phase advance (90 degrees) when the stimulus frequency was below 15 Hz. The latency of this reflexly activated $D_s$ spike decreased when the stimulus frequency was increased from 1-20 Hz (figure 11 ). Wilson (1965) also found that during forced movements of the whole leg, the evoked proprioceptive leg reflexes followed input

frequencies at more than 20 Hz without a noticeable phase shift. The constant phase advance of the $D_s$ reflex response at different stimulus frequencies is probably caused by the velocity sensitive properties of the hair plate sensilla. As the stimulus frequency increased, the discharge frequency of the hair plate sensilla (both type I and type II) within each stimulus cycle increased and more type I sensilla are recruited to respond (type I sensilla have velocity thresholds). Consequently as the stimulus frequency increased, the amplitude of the afferent input also increased. The increase in amplitude of the sensory input will cause a larger and faster rise of the membrane potential of motoneuron $D_s$ and hence will excite $D_s$ with a shorter latency. At 20 Hz of stimulus frequency, the latency for the first motor spike was 10 msec. Further increase in stimulus can not reduce this latency. This latency is apparently the fixed minimum latency required for the activation of the $D_s$ response when the hair sensilla of the hair plate are stimulated mechanically.

The inhibitory effects of the afferent activity from the hair plate affect both the tonic and bursting activity of the flexor motoneurons (figure 15,16). During rhythmic bursting activity of flexor motoneurons input from the hair plate can reset the rhythm of the bursts (figure 16 ). Previous studies have shown that the flexor bursts are generated by a system of nonspiking interneurons (Pearson & Fourtner, 1975), and it will be shown by intracellular studies in chapter 4 that the hair plate afferents excite and inhibit a number of nonspiking neurons. These results suggest that the afferent activity of the hair plate has direct influence on the system of interneurons producing the

flexor bursts. The inhibitory effect of the hair plate afferent on the tonic activity of the flexor motoneurons 5 and 6 is of short latency. An approximate estimate of the latency inhibitory pathway was less than 10 msec. This inhibitory effect is probably mediated via a disynaptic pathway (see chapter 4).

(ii) *Function of the trochanteral hair plate during walking*

Following the removal of the trochanteral hair plate in the leg of the cockroach, the operated leg oversteps during walking due to exaggerated flexion movement of the femur. Correspondingly, the activity of the flexor bursts are more intense and the duration of the bursts are prolonged. These changes in motor activity of the flexor burst can be a direct consequence of the removal of the inhibitory effect of the hair plate afferents on the flexor motoneurons and/or the system of interneurons producing the flexor bursts. In a normal animal, femur flexion presumably excites the hair plate afferents near the end of protraction and this afferent activity reduces the intensity and duration of the flexor burst during every cycle of walking. The fact that the animal oversteps following the removal of the hair plate suggests that the system of interneurons producing the flexor burst always generates a flexor burst with prolonged duration and sensory feedback is required to terminate the flexor burst in every cycle of walking. A similar function of the phasic sensory feedback is described in the masticatory system of the snail. During feeding activity, the input from mechanoreceptors inhibits the activity of the protractor motoneurons which otherwise might continue to fire into the retraction phase (Kater & Rowell, 1973). In the locust flight system,

inputs from the wing stretch receptors excited during wing elevation inhibit the elevator motoneurons and excite the depressor motoneurons. These reflex pathways may therefore function to limit the amplitude of wing elevation (Burrows, 1975). It is perhaps significant that in all these motor systems (walking, mastication and flight) reflexes function to terminate the motor activity underlying the phase of movement in which there is unlikely to be any variation in load. A similar control for the position of end flexion in the forelimb of the dog has also been described (Orlovsky & Shik, 1965). It was found that during walking, the elbow of the dog always flexed to a definite angle irrespective of the initial angle when flexion began.

The advantages of utilizing the feedback from the hair plate afferents to terminate the centrally generated flexor burst are not immediately apparent. Possibly it is involved in preventing a decrease in amplitude of femur flexion with muscle fatigue. Without inhibitory feedback from the hair plate to the flexor motoneurons and/or the flexor burst generating system any fatique in the flexor muscles would reduce the amplitude of the flexion movement. However if the hair plate functions to terminate the centrally generated flexor burst and is activated at a fixed angle of flexion of the femur (see discussion in chapter 1 ), then the amplitude of the femur movement will remain constant even with fatique of the flexor muscles. Another possible function of the negative feedback from the hair plate to the flexor motoneurons in the hind and middle legs is to prevent mechanical interference of these legs with the adjacent leg during walking.

Since the hair sensilla of the hair plate are activated near

the end of the protraction phase of leg movement in a walking animal, the excitatory connection of the afferents of the hair plate on the extensor motoneuron $D_s$ could be important in the initiation of the extensor burst. Accordingly it can be assumed that if the hair plate is removed, the occurrence of the extensor burst in a cycle will be delayed, causing a longer interval between the end of a flexor burst and the start of an extensor burst. However, this expected result was not observed. Probably other afferent channels (e.g. input from campaniform sensilla) are involved in initiating the extensor burst and the removal of one channel may not produce significant changes. Therefore the function of the hair plate in initiating the extensor burst during walking cannot be excluded by our negative finding. The excitatory pathway from the hair plate to the extensor motoneuron $D_s$ may also function to stabilize leg posture during standing. Any external disturbance which tends to flex the femur will excite the hair plate and hence reflexly increase the level of activity in the femur extensor motoneuron $D_s$. Consequently an extension force will be developed to resist the imposed flexion.

# CHAPTER 4

## INTRACELLULAR STUDIES

### 4.1. INTRODUCTION

(i) *Impulse conduction in the terminal branches*

Axon terminals of the motoneurons and sensory neurons branch profusedly before forming synaptic connections with the next cell (Burrows, 1975; McIntyre, 1974; Usherwood, 1967). Theoretical considerations have shown that an increase in input conductance can occur at the branch points of the nerve fiber which will reduce the safety factor for the propagation of impulse past this point (Goldstein & Rall, 1974). Failure of impulses to invade some terminals of the motor axon in the neuromuscular junctions of both vertebrates and invertebrates has been reported (Krnjevic & Miledi, 1959; Parnas, 1972; Hatt & Smith, 1975) and it has been demonstrated in the invertebrate preparation that this failure of impulse occurred at the branch point in the motor axon terminal (Grossman, Spira & Parnas, 1973). The possibility that the branch points of the sensory terminals may influence impulse conduction has been suggested by a number of previous studies (Chung, Raymond & Lettvin, 1970; Dun, 1951; Rall, 1964; Wall, Lettvin, McCullock & Pitts, 1956). There is strong evidence that even relatively small increases in $pCO_2$ may cause a block of impulse conduction in the sensory terminals (Morris, 1971a; 1971b). However, at the present time, impulse conduction block in the sensory axon terminals has not been demonstrated directly by intracellular recording techniques. I have been able to record the intracellular activity of the sensory terminals and one of the aims of the work in this chapter has been to demonstrate directly the phenomenon of conduction block of impulses in the terminal branches.

(ii) *Postsynaptic potentials evoked in motoneurons*

The recent use of intracellular recording techniques has led to the identification of monosynaptic excitatory pathways from the sensory afferents onto motoneurons in the locust flight system (Burrows, 1975) and crab eye movement system (Sandeman 1969; Silvey 1974). With the lack of studies in the majority of other arthropod systems the extent of the existence of monosynaptic reflex pathways cannot yet be assessed, and it is certainly premature to propose a general rule that most reflexes are mediated via interneurons (Burrows & Horridge, 1974). In the previous chapter it was demonstrated, using extracellular recording techniques, a short latency excitatory connection from trochanteral hair plate afferents to the slow coxal depressor motoneuron $D_s$ in the cockroach metathoracic leg. The results reported in this chapter show that this connection is monosynaptic and transmission across it is mediated chemically.

The only detailed intracellular study of an inhibitory reflex pathway in arthropods is that by Burrows (1975) on the locust flight system, where he showed that impulses in wing stretch receptor afferents evoke fairly short latency (4-6 msec.) inhibitory postsynaptic potentials IPSPs, in wing elevator motoneurons. He concluded that this inhibitory connection is probably monosynaptic. However, from the published data, the possibility that the IPSPs were elicited via a polysynaptic pathway was not tested and excluded. In Chapter 2 it is shown that activity in the trochanteral hair plate afferents produces a short latency inhibition of tonic activity in leg flexor motoneurons of the cockroach. Intracellular studies were performed to determine whether this

inhibitory effect was mediated monosynaptically or polysynaptically. Although the data does not exclude the possibility of a monosynaptic inhibitory connection, it is more probable that IPSPs elicited in flexor motoneurons and some unidentified interneurons, in response to activity in trochanteral hair plate afferents are produced via a pathway containing a non-spiking inhibitory interneuron.

## 4.2. METHODS

### (i) *The preparation*

Experiments were performed on male and female cockroaches, *Periplaneta americana*. The experimental arrangement is shown in Figure 19 In most preparations the animal's left metathoracic leg was rotated to expose the dorsal surface of the coxa, and the nerve 6Br4 to the left posterior coxal levator muscles was cut close to the levator muscles and retracted medially. This allowed recordings to be made from the motor axons in this nerve with the ventral surface of the coxa uppermost (figure 19). In other preparations nerve 6Br4 was left intact.

The animal was mounted in wax ventral side up on a cork board. The coxa of the left leg was held rigidly in wax. An insect pin positioned in the anterior border of the femur kept the femur in a slightly extended position so that the trochanteral hair plate was exposed. Extension movements of the femur was possible when the leg was fixed in this manner. The cuticle above the metathoracic ganglion and nerve 5 was removed together with the underlying fat deposits. The exposed ganglion was constantly moistened by periodic application of cockroach saline (215 mM sodium chloride, 3.1 mM potassium chloride, 1.8 mM calcium chloride, 2.0 mM sodium bicarbonate, 0.1 mM sodium

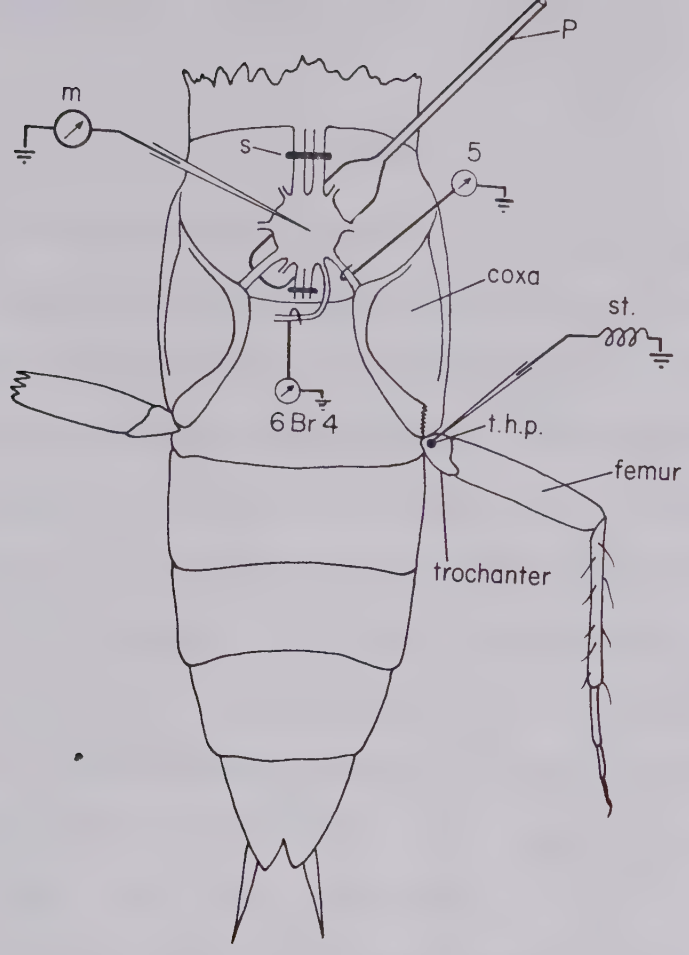


Figure 19. Schematic diagram showing the experimental preparation and the arrangement of recording and stimulating electrodes. The animal was fixed ventral side up on a cork board and a stainless steel plate, p, placed under the exposed metathoracic ganglion. Staples, s, were placed around the anterior and posterior connectives to hold the ganglion firmly on the plate. Monopolar extracellular recording electrodes were placed on nerve 5 and nerve 6Br4 (the latter being positioned after dissecting nerve 6Br4 from the dorsal side of the coxa). The trochanteral hair plate, t.h.p., was exposed by extending the femur and removing a cuticular flange at the distal end of the coxa. A fine stimulating electrode, st, was placed on the hair plate. The ganglion was penetrated by an intracellular recording microelectrode, m, on the side ipsilateral to the stimulating electrode.

phosphate monobasic). A stainless steel platform (p) was positioned under the ganglion and staples (s) placed around the anterior and posterior connectives. This procedure held the ganglion reasonably firm. Extracellular recording electrodes were placed either on nerve 6Br4 or in the coxal levator muscles to monitor the activity in the flexor motor axons to the posterior coxal levator muscles. Recording electrodes were also placed on nerve 5 to monitor the afferent input from the hair plate and activity in the slow motoneuron $D_s$ to the coxal depressor muscles. Monopolar silver electrodes were used for all nerve recordings and 75μ copper wires insulated to the tip were used for muscle recordings. The hair plate afferents were either excited by mechanical stimulation (see chapter 2 or by the application of 0.05 msec. current pulses through a sharp insect pin to the base of the hairs in the hair plate. By carefully placing the stimulating electrode and finely adjusting the current, single afferents from the hair plate could be excited. Sychronous activation of more than one afferents was achieved by using larger stimulus current (see chapter 2 ).

(ii) *Intracellular recording*

Intracellular recordings were made with 2.0 M potassium citrate - filled microelectrodes. The glass tubing (pyrex glass tubing 0.86 mm. OD and 0.51 mm. ID purchased form Drummond Scientific, Broomall, Pa.) was threaded with four strands of fiber glass before pulling. The fiber glass strands not only allow the immediate filling of the electrodes (Taski, Tsukahara, Ito, Wayner & Yu, 1968) but also increased the mechanical strength of the tips (Pearson & Fourtner, 1975). All

electrodes were inspected microscopically before use and rejected if the tip showed any sudden changes in diameter. The best tips were ones in which the diameter decreased gradually so that the taper of the tip was constant. The electrode resistance was between 20 and 30 Mohms.

The ganglion was prevented from drying during the experiment by placing a small wax ring around it and saline was retained within the wax ring. The connective sheath of the ganglion was softened by treating the ganglion with a 0.1% pronase-in-saline solution for 10 minutes, then thoroughly washed with saline. The procedure adopted to penetrate the sheath with the microelectrode was as follows. The electrode was very slowly lowered until its tip lightly touched the ganglion. This was indicated by and increase in the recorded electrode noise. The electrode holder was then gently and regularly tapped with a finger while the electrode was lowered still further. Penetration of the sheath was indicated by a sudden negative shift of 70-90 mV, followed by the sudden return of the potential to zero. This negative shift is presumably due to the electrode tip entering glial cells on the periphery of the ganglion. To successfully penetrate the neurites within the neuropil it was necessary to continue the light tapping of the electrode holder. Penetration of a neurite was indicated by a sudden negative potential shift of between 30 and 70 mV, by the appearance of synaptic activity, and often by the appearance of spikes.

The amplifier used for these intracellular studies was a WP Instruments M-4 electrometer. A bridge circuit in this instrument allowed currents to be passed into the cells and the resultant changes

in membrane potential to be observed. The magnitude of the current injected was monitored by recording the potential drop across a 100 Kohm resistor in the ground return. In some experiments the microelectrode was also used to moniter the extracellular activity in the afferent terminal branches in the ganglion.

(iii) *Staining of the hair plate afferents*

Preliminary studies were performed to define anatomically the geometry of the hair plate afferent fibers within the ganglion. Cobalt chloride was allowed to diffuse up the afferent axons, then the preparation was treated with amonium sulphide to form a black precipitate of cobalt sulphide.

The cockroach was fixed ventral side up in wax on a cork-board. All the legs were firmly fixed and no movement was allowed. The trochanteral hair plate of the metathoracic leg was removed by a pair of fine forceps. The dendrites of the sensory cells of the hair sensilla were thus exposed. A drop of concentrated solution of cobalt chloride was then applied to the hole in the cuticle where the hair plate was situated prior to its removal. Colbalt chloride then could diffuse up the exposed end of the dendrites of the sensory cells. The preparation was kept in a humid closed chamber at room temperature for 24 hours. The metathoracic ganglion was then removed from the preparation and was washed in saline for 30 minutes. The washed preparation was then bathed in 0.05% ammonium sulphide in saline solution for 30 minutes. After a 30 minutes wash in saline, the ganglion was dehydrated in alcohols and cleared in creosote. By applying this procedure, the

hair plate afferents and their terminal branches in three ganglia had been lightly stained. It seemed that modifications in the procedure (e.g. concentration of colbalt chloride, time allowed for back diffusion of colbalt chloride) are required before good stained preparations can be obtained.

## 4.3. RESULTS

### (i) *Recordings from afferent terminal branches*

Prior to the penetration of the afferent terminal branch by the microelectrode, extracellular activity of the terminal branch was recorded by the electrode. Figure 20 shows the record obtained when the microelectrode was in close proximity of the afferent terminal branch. The recorded potential showed a biphasic (positive-negative) shape indicating that the terminal branch was invaded by the action potential (Eccles, 1964; Hubbard and Schmidt, 1963). As the microelectrode was lowered further, the magnitude of the recorded biphasic potential became larger, suggesting the microelectrode was now closer to the terminal. With still further lowering prior to the penetration of the terminal branch by the microelectrode, the shape of the extracellularly recorded potential abruptly changed from biphasic to monophasic (positive only), the negative peak of the previously recorded biphasic potential disappeared (Figure 20b). Monophasic positive potentials were also recorded in the presynaptic terminals of the crustacean neuromuscular junction (Dudel & Kuffler, 1961). It was suggested that the shape of the recorded potential (monophasic, positive) indicated that the presynaptic action potential failed to invade the terminal branch (Dudel & Kuffler, 1961; Eccles, 1964).

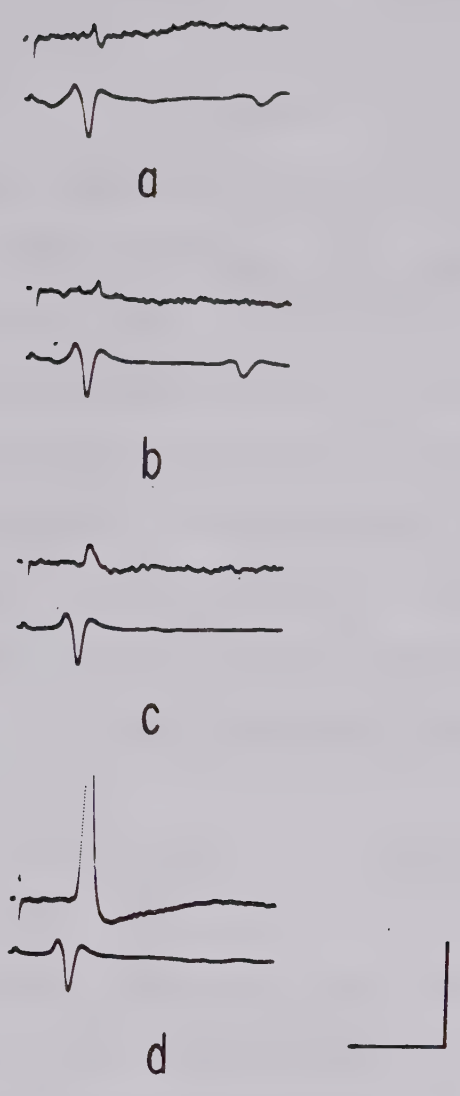


Figure 20. Extracellular recordings from terminal branch of the trochanteral hair plate afferent by a microelectrode inserted in the ganglion. Top traces - extracellular recordings from terminal branch; bottom traces - nerve 5 records. The electrode is inserted gradually deeper into the ganglion from (a)-(d). Notice that the extracellularly recorded potential in the terminal branch changed shape from biphasic (positive-negative) in (a) to monophasic in (b). In (d), the electrode is made to penetrate the branch and the intracellular impulse is recorded. Calibrations, top trace of each record: amplitude - 2mV; time - 4 msec.

The change in shape of the extracellular recorded potential in the afferent terminal branch suggests that a blockage of the action potential occurred when the microelectrode was placed very close to the terminal branch. Probably, the pressure of the microelectrode on the terminal branch caused the block. After penetration of the terminal branch by the microelectrode, the intracellular recorded spike was within the range of 20-40 mv and the peak was never positive. The characteristics of these spikes are similar to those recorded from sensory afferent terminals in other animals (Kenedy, Calabrese, & Wine, 1974; Koketsu, 1959).

The latency of the afferent terminal spike following the axon spike recorded from nerve 5 was in the range of 0.5 to 0.8 msec. in different preparations (figure 21 ). The latency was measured from the negative peak of the triphasic extracellular record to the positive peak of the afferent terminal spike. Both these points could be accurately identified in our records. Since the negative peak of the triphasic record from nerve 5 corresponds to the time when the peak of the action potential is passing the recording site (Stein and Pearson, 1971), this measurement of latency allowed the caculation of the average conduction velocity in the afferents. The distance between the point of recording on nerve 5 and the site of recording within the ganglion was approximately 2 mm, thus the average conduction velocity of the action potentials in the hair plate afferents was between 2.5 and 4 meters/sec. This estimate of the range of conduction velocities of the action potentials in the hair plate afferents is very close to

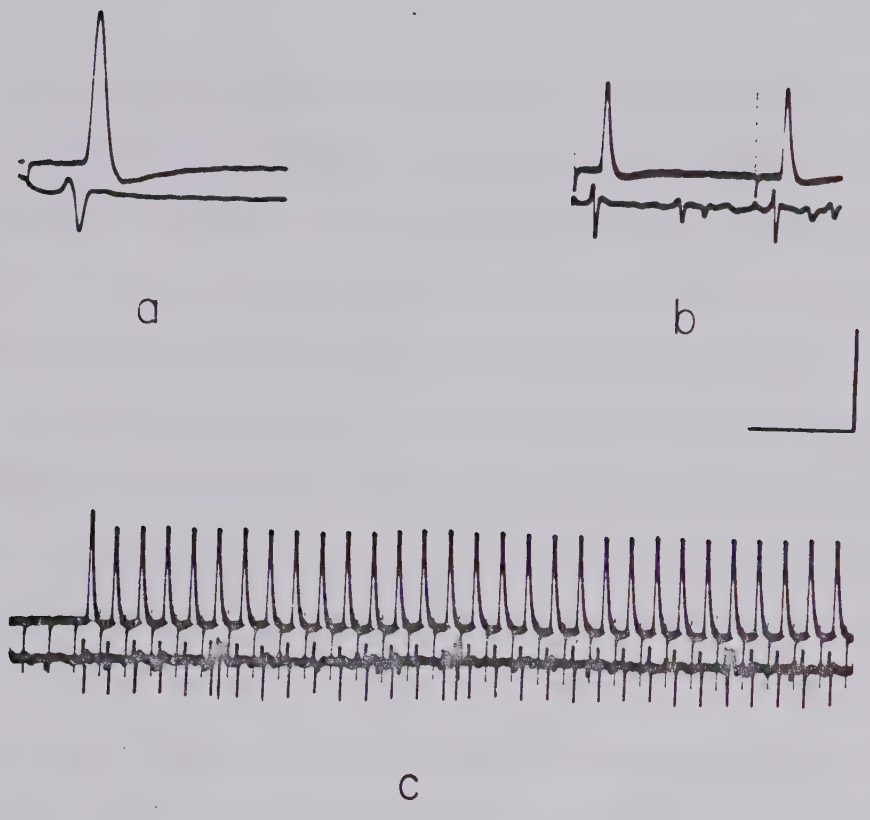


Figure 21. Intracellular recordings from terminal branches of trochanteral hair plate afferents. Top traces - intracellular records; bottom traces - nerve 5 records. (a) the extracellular potential recorded from the axon of a single afferent (bottom) is followed shortly by an action potential in one of its terminal branches (top). Note that there is no prepotential preceding the terminal spike. (b) and (c) are records from another preparation showing that the terminal spikes follow 1:1 high frequency stimulation. In (c) the stimulating frequency was 200/second. Calibrations: terminal spike amplitude - 20 MV; time - (a) 4 msec., (b) 8 msec., (c) 20 msec.

that (3.5 - 5 meters/sec.) reported in chapter 2. Having obtained recordings from the terminal branches of the hair plate afferents, it was then possible to determine more accurately the times of transmission of information from these afferents to other neurons within the ganglion.

In experiments designed to demonstrate conduction block in the afferent terminal branches double electrical stimuli were applied to excite the hair plate sensillum. Following the absolute refractory period due to the first impulse, there was a gradual recovery of the excitability of the axon and its terminal branches (relative refractory period) (Stein, 1967; Fuortes, & Mantegazzini, 1962). During this time it was possible to evoke a second impulse from the sensillum. The ability of the second impulse to invade the terminals at various intervals after the first impulse was examined. The double shock procedure was used to detect low safety factor regions for impulse conduction in the motoneurons in the cat (Brock, Coombs, & Eccles, 1953) and giant fibers in the cockroach (Parnas, Spira, Werman & Bergman, 1969).

Conduction block in the afferent terminal branches was first indicated by recordings made of evoked postsynaptic events in a nonspiking neuron. Nonspiking neurons were classified by their synaptic noise (Pearson & Fourtner, 1975) and the inability of applied current to evoke spike in them. Figure 22a shows the intracellular response recorded form a non-spiking neuron to a pair of afferent impulses. These impulses generated unitary EPSPs which summed. The delay between the extracellular activity of the afferent

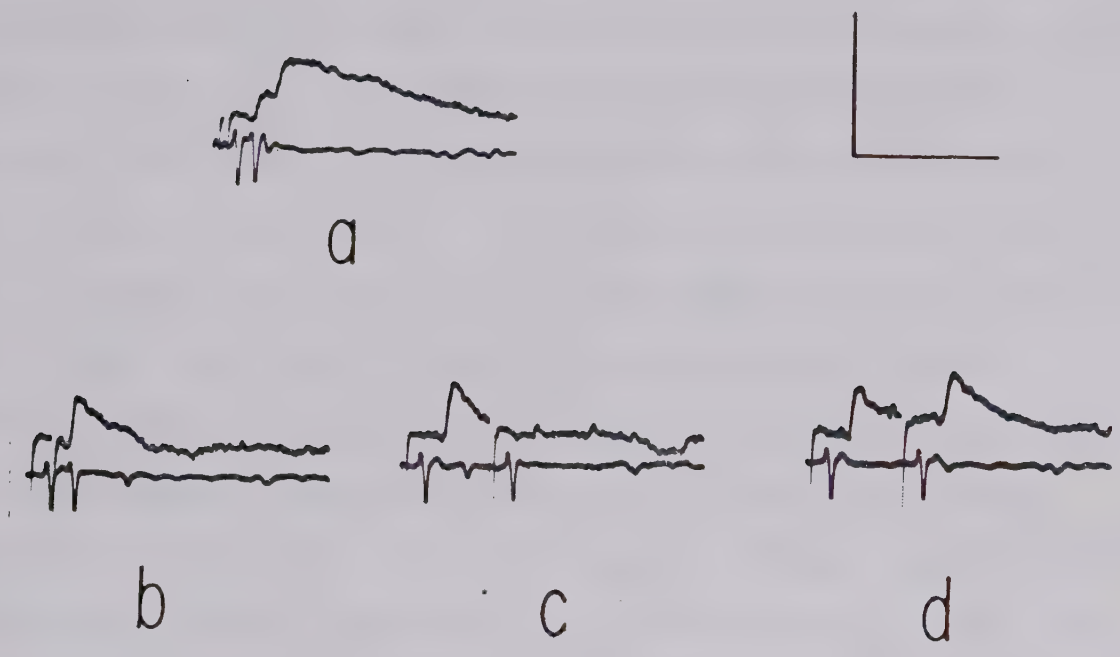


Figure 22. Records showing EPSPs recorded in a nonspiking neuron in response to electrical stimulation of trochanteral hair afferents. Top traces - intracellular records; bottom traces - nerve 5 records. (a) Double electrical stimuli were applied to one hair plate receptor, two evoked impulses were recorded by the electrodes placed on nerve 5. The EPSPs generated by the afferent impulses summed. (b-d) Another hair plate receptor was stimulated. A minimum interval between the two evoked afferent impulses was required (6 msec.) before the second afferent impulse generated an EPSP (d). Calibrations: Amplitude - 10 mV; Time - 10 msec.

impulse picked up in nerve 5 and the onset of EPSP was 1.1 msec. This value of delay time suggests that the EPSP was generated monosynaptically by afferent impulse (see following section on excitatory postsynaptic potentials). The stimulating electrode was then moved and placed to stimulate another hair receptor. The response of the same neuron to a pair of impulses evoked in the second afferent fiber is shown in figure 22b-d. In this case, only the first of two closely spaced impulses gnerated an EPSP. The delay measured between the recorded extracellular activity of the first impulse and the onset of the EPSP was 1.1 msec. This measured delay is the same as that recorded in figure 22a. The second afferent fiber therefore also established a monosynaptic connection with the neuron. When the test interval was increased, eventually an EPSP was generated by the second afferent impulse. The minimum-response-interval for the second impulse to generate an EPSP was 6 msec. (figure 22d). When the test intervals were longer than 6 msec., the second impulse always generate an EPSP. Two possible mechanism could have caused the failure of the second impulse to generate an EPSP. The impulse may have failed to invade the terminals or it may have invaded the terminals but failed to cause transmitter release. To distinguish between the two possibilities, intracellular recordings were made in the terminal branches of the afferent fiber. The criteria used for identifying a record from the terminal branch of a hair plate afferent were : (1) the spikes elicited in them by stimulation of the hair plate were of short duration (approx. 1.5 msec) and without a prepotential, (2) the spikes followed the impulse recorded extracellularly from the afferent axon in nerve 5 with short latency, and (3) the lack of any spontaneous synaptic activity. One further reason for assuming that these recordings were made from the terminal branches of

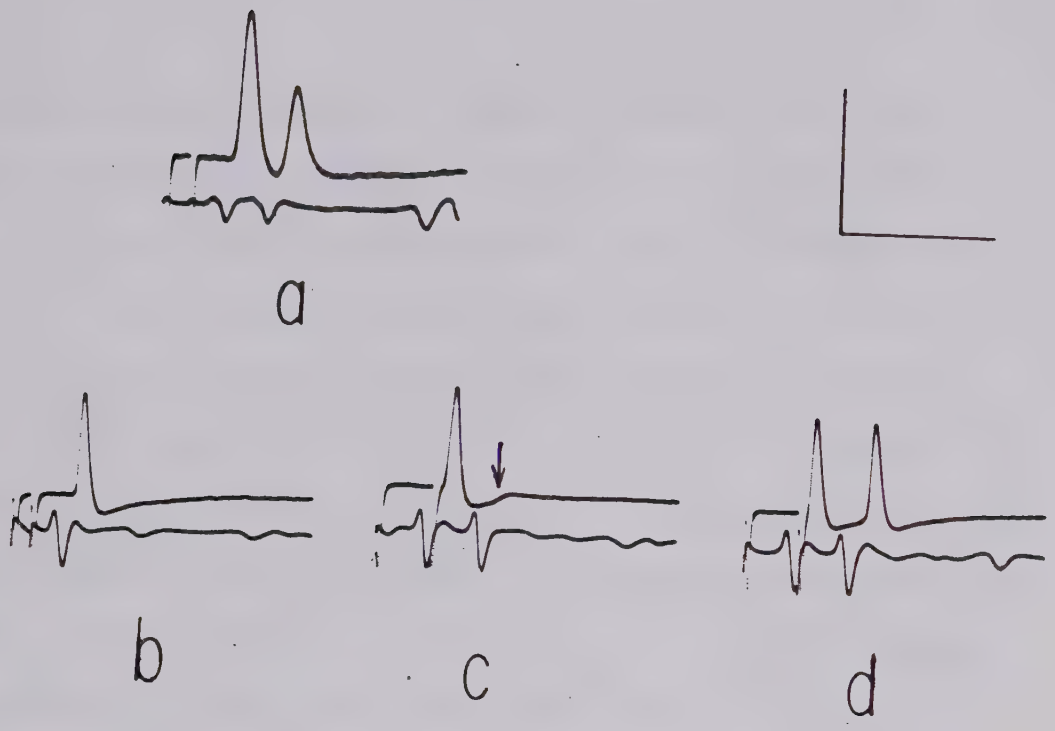


Figure 23. Intracellular recordings from terminal branches of trochanteral hair afferents. Top traces - intracellular records; bottom traces - nerve 5 records. (a) Impulses evoked in one hair plate receptor invaded the terminal regardless of the test interval. (b-c) In the terminal of another afferent a minimum interval was required (2 msec.) before the second impulse of a closely spaced pair invaded the terminal (d). Notice a small prepotential (arrow, c) that appears when the second impulse failed to invade the terminal. In (b) only one impulse was evoked from the hair receptor by the double stimuli. Probably the second stimuli was applied during the absolute refractory period of the receptor. Calibrations: Amplitude - 25 mV; time - 5 msec.

the afferents is that preliminary anatomical studies using cobalt staining techniques have shown that some of the terminal branches of the hair plate afferents are located in the region of the ganglion from which these recordings were made (see section iii of method). Figure 23a shows that for one afferent fiber, the impulse invaded the terminal branch regardless of the duration of the test interval. In this case, the second impulse recorded intracellularly is smaller than the first impulse because the second impulse was generated during the relative refractory period. The results recorded from this afferent corresponded to the postsynaptic recording illustrated in figure 22a where EPSPs were always generated by the afferent impulses regardless of the duration of the test interval. Recordings were then made in the terminals of other afferent fibers from the hair plate. Figures 23b-d show the intracellular record obtained in one terminal where a minimum test interval was required before the second impulse invaded the terminal. When the test intervals were shorter than 2 msec., the second impulse only generated a small positive potential at the site of recording (Figure 23c). This small potential was probably caused by the electrotonic spread of the afferent impulse from the site prior to its failure.Figure 23d shows that when the test intervals were longer than 2 msec., the second impulse always invaded the terminal. It can be observed that at this minimum-response-interval the size of the second impulse had almost reached the size of the first impulse, indicating that by the time the second impulse invaded the terminal, the terminal had almost totally recovered from its refractoriness.

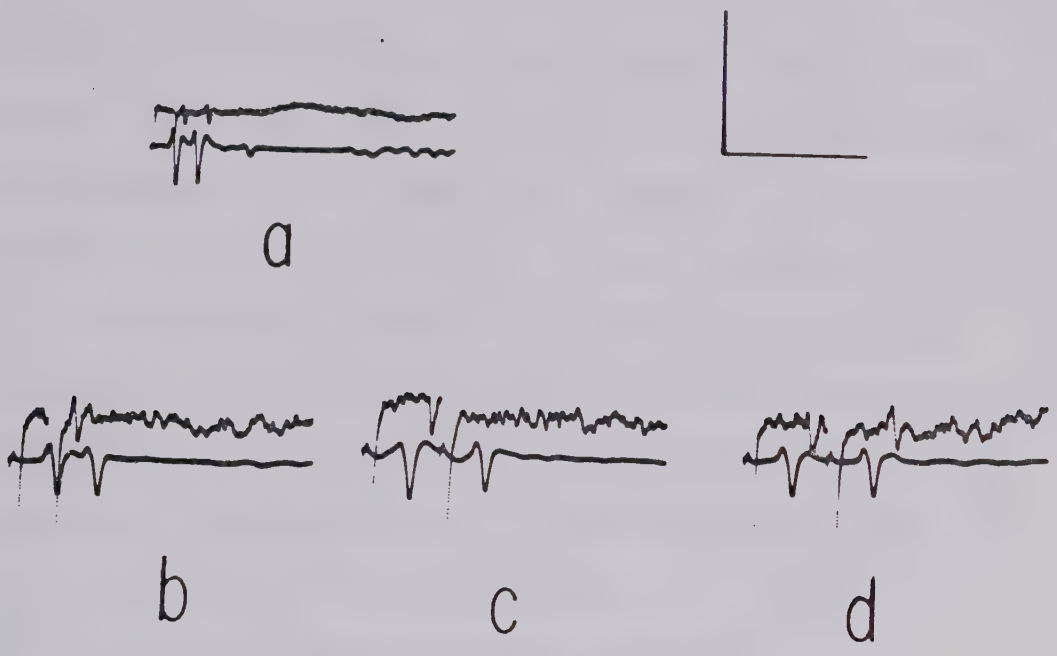


Figure 24. Extracellular recordings from terminal branches of trochanteral hair plate afferents. Top traces - extracellular records obtained by microelectrodes placed close to the terminal branches; bottom traces - nerve 5 records. (a) Invasion of the terminal by two closely spaced afferent impulses evoked from a hair plate receptor. (b-d) For the terminal of another afferent, a minimum interval (3 msec.) was required before the second impulse of a closely spaced pair invaded the terminal (d). The breaks in the extracellular records (top trace b-d) are caused by the stimulus artifact. Calibrations: Amplitude - (a) 5 mV, (b-d) 2.5 mV; time - (a) 10 msec., (b-d) 5 msec.

Intracellular recording from these fine branches of nerve fiber inevitably caused damage to them. One indication of this is that stable recordings rarely lasted longer than 15 minutes. In order to investigate whether conduction failure also occurs in undamaged terminals, the extracellular activity of the terminals was recorded. Figure  illustrates the recordings obtained by a microelectrode placed close to the afferent terminals. Two impulses invaded the terminal of one afferent (figure 24a) regardless of the interval between them. For the other afferent (figure 24b-d) a minimum interval of 3 msec. was required before the second impulse invaded the terminal.

(ii) *Excitatory postsynaptic potentials*

Electrical stimulation of the hair plate afferents evoked an excitatory postsynaptic potential, EPSP, in at least two motoneurons giving extension movements of the femur and a number of unidentified nonspiking neurons (figure25 see Pearson and Fourtner, 1975, for the criteria used for classifying a neuron as a nonspiking neuron). The two extensor motoneurons found to be excited by the hair plate afferents were 1) the slow motoneuron to the coxal depressor muscles, motoneuron $D_s$, and 2) a fast motoneuron to the main coxal depressor muscles with its axon in nerve 4.

To record from the neurites of motoneuron $D_s$, microelectrode penetrations of the neuropil were made slightly posterior to lateral nerve trunk 3 and from 150 to 250 microns from the midline. In a previous study it has been shown that a number of prominent neurites of this motoneuron are located in this region (Pearson and Fourtner, 1975).

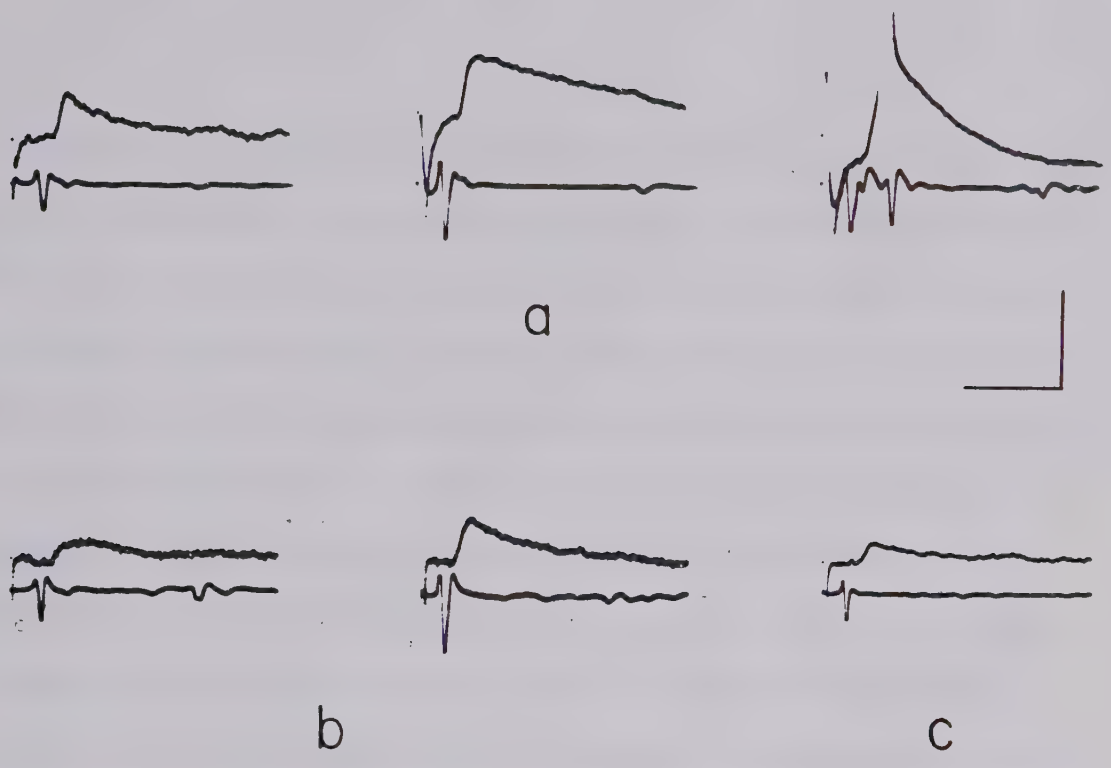


Figure 25. EPSPs recorded in extensor motoneurons (a and b) and a non-spiking neuron (c) in response to electrical stimulation of trochanteral hair plate afferents. Top traces - intracellular records; bottom traces - nerve 5 records. (a) EPSPs in motoneuron $D_s$: left - EPSP in response to activity in a single afferent; middle - increase in the amplitude of the EPSP upon synchronous activation of a number of afferents; right - a large EPSP elicits an action potential in motoneuron $D_s$ (this action potential can be seen as the second spike in the extracellular record from nerve 5). (b) EPSPs in a fast extensor motoneuron: left - EPSP in response to activity in a single afferent; right - increase in amplitude of the EPSP upon synchronous activation of a number of afferents. Calibration: amplitude EPSPs - 4 mV; time - 8 msec.

Since motoneuron $D_s$ is the only motoneuron with an axon in nerve 5 to be readily activated by hair plate stimulation, the criterion used for establishing that recordings were made intracellularly from this motoneuron was that stimulation of the hair plate afferents evoked a short latency spike in the impaled neuron and this spike was correlated 1:1 with spikes recorded from a single motor axon in nerve 5 (the axon of motoneuron $D_s$ is in nerve 5). To confirm that this criterion alone is sufficient for identifying motoneuron $D_s$, extracellular recordings were made from the coxal depressor muscle in which the excitatory junctional potentials evoked in these muscles by motoneuron $D_s$ can be unambiguously identified (Pearson, 1972). A fast extensor motoneuron with an axon in nerve 4 was identified by the 1:1 correspondence of intracellular spikes with phasic extension movements of the femur and the absence of a spike in the extracellular record from nerve 5. Since there are a number of fast axons in nerve 4 to the coxal depressor muscle, it was not certain that the same fast extensor was penetrated in different preparations. No attempt was made to physiologically or anatomically identify the nonspiking neurons from which EPSPs were recorded. Since these interneurons were encountered in different areas of the dorsolateral region of the ganglion, it is probable that a significant number of interneurons receive excitatory input from the hair plate afferents.

An important observation was that in repeated penetrations in the same preparation different neurons were encountered in which the same afferent fiber produced EPSPs. This observation demonstrates that

single hair plate afferents can simultaneously exite both extensor motoneurons and certain nonspiking neurons.

The evoked EPSPs in the slow and fast extensor motoneurons and the unidentified neurons were very similar as regards their amplitude, time course and latency. Consequently, in the following description of these characteristics of the EPSPs, a distinction will not always be made between the EPSPs recorded in different Neurons.

The EPSPs produced by a single afferent had an amplitude of between 1.0 and 2.0 mV. There was no obvious correlation between the amplitude of the spikes recorded extracellularly on nerve 5 from single afferents (which gives an indication of the diameter of the afferent; Pearson et al., 1970) and the EPSP amplitude. Stimulation of more than one hair plate afferent led to a larger EPSP due to the summation of the individual EPSPs (figure 25). An EPSP of more than a few millivolts usually initiated an action potential in motoneuron $D_s$ (figure 25), while synchronous activation of many hair plate afferents only rarely initiated a spike in the fast extensor motoneuron.

The duration of the EPSPs was between 10 and 20 msec. and the rise time approximately 2 msec. The time course of these EPSPs is similar to that observed in other neurons of the cockroach (Callec et al., 1971).

The latency of the EPSPs measured from the negative peak of the triphasic potential recorded from the afferent axon in nerve 5 was extremely constant for repeated stimulation in a single preparation (figure 26), and within the range of 0.9 and 1.6 msec. in different

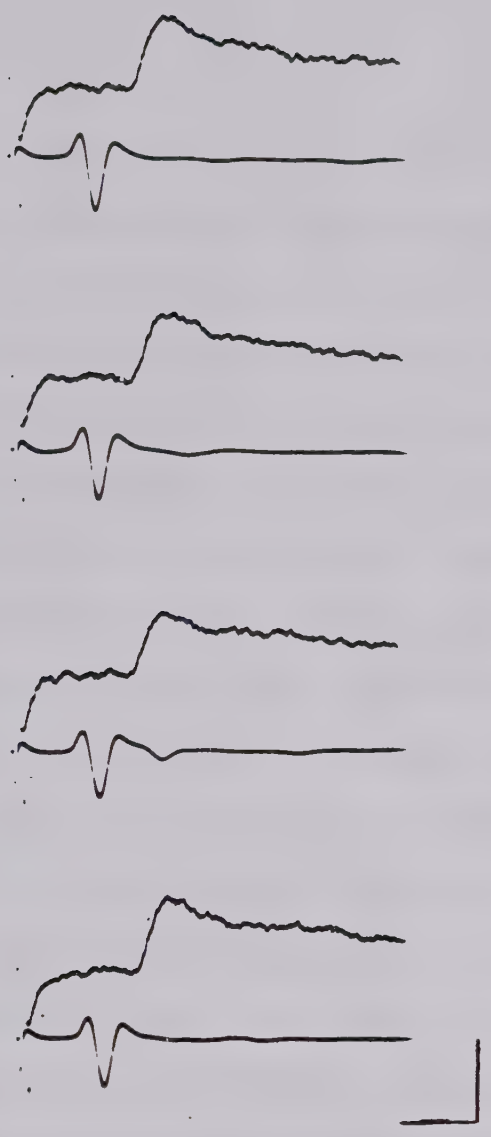

Figure 26. Records showing the constant latency of the EPSPs elicited in motoneuron $D_s$ (top traces) by activity in a single hair plate afferent. Bottom traces - nerve 5 record of afferent activity. The four sets of records shown in this figure are from four consecutive stimulations of the hair plate afferent. Calibration: amplitude EPSPs - 2 mV; time - 2 msec.

preparations (figure 27). Table1 lists the mean value of the latency measured in the extensor motoneurons and the nonspiking interneurons in different preparations. From this it can be seen that for all these neurons the mean latency was very similar and slightly greater than 1 msec. Allowing for the conduction time of the afferent impulses into the ganglion (which is approximately 0.6 msec.; see figure 27) the transmission time from the afferent terminal spike to the initiation of the EPSPs is clearly less than 1 msec. In two preparations it was possible to directly measure this delay since I was fortunate enough to first record the EPSP elicited by a spike in a single afferent then on a subsequent penetration of the ganglion record intracellularly from the terminal branch of the same afferent (figure 28). The latency between the peak of the afferent terminal spike and the beginning of the EPSP in both preparations was 0.4 msec. This small but significant value of the central transmission delay indicates that the EPSPs are elicited via a monosynaptic connection from the hair plate afferents and that transmission across this synapse is chemically mediated.
A further demonstration that there is an appreciable delay between the afferent terminal spike and the initiation of the EPSPs is seen by comparing the latencies of these two events in all preparations in which they were recorded (figure 27). The mean value of the peak of the terminal spike was 0.62 msec., while the mean value of the latency to the beginning of the EPSP was 1.17 msec. These observations argue against the possibility that transmission between the hair plate afferents and central neurons is via an electrical synaptic junction.

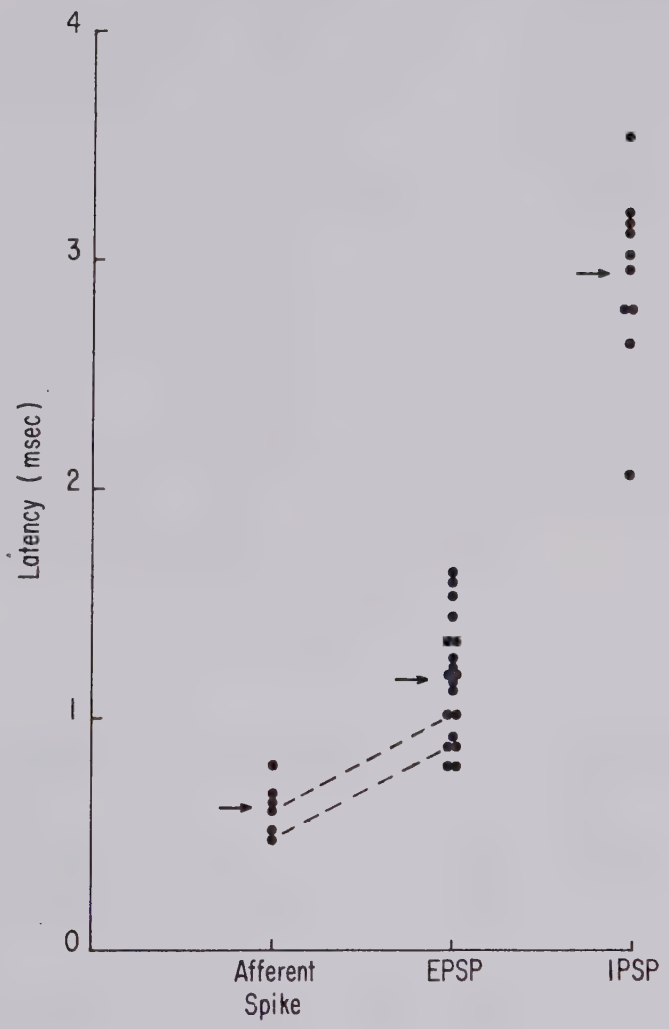


Figure 27. Diagram showing the latencies of the afferent terminal spikes, the EPSPs and the IPSPs measured for all preparations. The latencies were measured from the negative peak of the extracellular record of afferent activity in nerve 5 to the peak of the afferent terminal spikes and to the beginning of the postsynaptic potentials. The arrow to the left of each group of data points shows the mean latency for that group. The two dotted lines connect the data points obtained in two preparations in which an EPSP was first recorded in response to activity in a single afferent then on a subsequent penetration of the ganglion the terminal of the same afferent was impaled. In both cases the delay between the peak of the terminal spike and the beginning of the EPSP was 0.4 msec.

TABLE 1

Mean latencies in milliseconds of the afferent terminal spikes, EPSPs and IPSPs. The number of observations is bracketed.

| Afferent terminal spikes | EPSPs | | | IPSPs | | |
|---|---|---|---|---|---|---|
| 0.62 (6) | Motoneuron $D_s$ | 1.24 | (4) | Flexor motoneurons | 2.81 | (3) |
| | Fast extensor | 1.02 | (2) | Other | 3.00 | (7) |
| | Other | 1.17 | (15) | | | |
| | Total | 1.17 | (21) | Total | 2.95 | (10) |

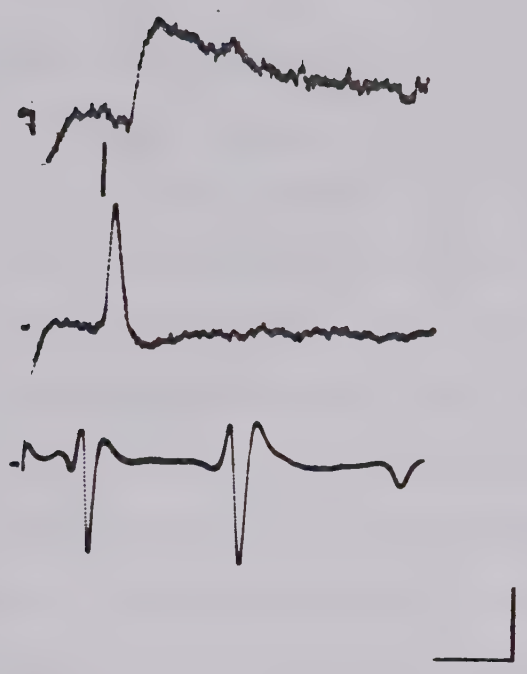

Figure 28. Records showing the delay between an afferent terminal spike (middle trace) and the beginning of an EPSP in a fast extensor motoneuron (top trace). The bottom trace is the nerve 5 record. The EPSP was evoked by an action potential in a single afferent and the terminal spike recorded in the same afferent on a subsequent penetration of the ganglion. The extracellular afferent spikes recorded from nerve 5 in the two sets of original records were aligned to show the delay between the beginning of the terminal spike (indicated by the short vertical line under the top record) and the beginning of the EPSP. Only one nerve 5 record is shown in this figure. The second spike in the nerve 5 record is from motoneuron $D_s$. Calibrations: EPSP amplitude - 2 mV; terminal spike amplitude - 10 mV; time - 4 msec.

The significant delay (approximately 0.4 msec.) between the peak of the spike in the terminal branches of the hair plate afferents and the beginning of the EPSPs indicates that transmission is chemically mediated. A second indication for chemically mediated transmission is that the duration of the EPSPs (10 to 20 msec.) is similar to the duration of other chemically evoked EPSPs in arthropods (Burrows, 1975; Callec et al., 1971; Zucker, 1972) and significantly longer than the duration of electrically evoked EPSPs (Zucker, 1972).

Two further tests for demonstating chemical transmission were carried out, namely the studying of the effect of 1) hyperpolarizing currents in the postsynaptic neuron on the amplitude of the EPSPs, and 2) bathing the ganglion in a solution containing a high concentration of magnesium. Figure29 shows that the passage of hyperpolarizing currents increases the amplitude of the EPSP, as is expected if transmission is mediated chemically. The effects of large depolarizing currents in motoneurons cannot be studied since the generation of action potentials by the depolarizing current obscured the evoked EPSPs. In nonspiking neurons depolarizing currents decreased the EPSP amplitude but in none of these neurons could sufficient current be passed to reverse the EPSP. By itself, the observation of an increased EPSP amplitude by a hyperpolarizing current does not conclusively prove that transmission is via a chemical transmitter since the imposed currents could alter the passive membrane resistance (Nichols and Purves, 1970). Nonetheless, this finding, together with the significant synaptic delay and long duration of the EPSP, strongly argues in favor

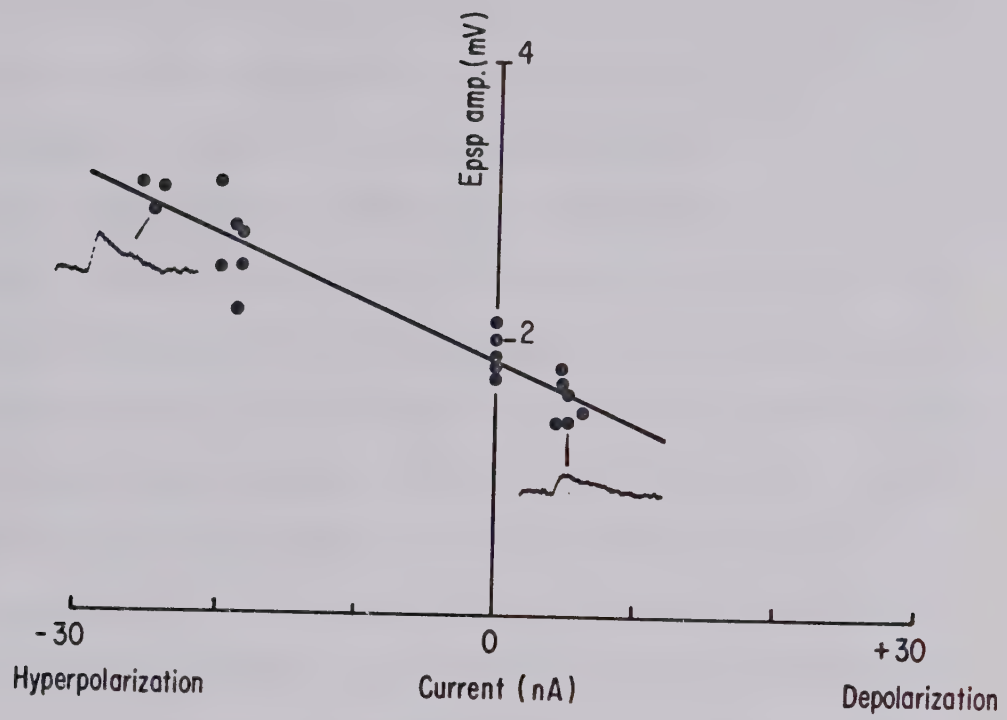


Figure 29. Effect of hyperpolarizing and depolarizing currents on the amplitude of the EPSPs evoked by stimulation of the trochanteral hair plate afferents. Fast extensor motoneuron.

of chemically mediated transmission.

A conventional technique to demonstrate chemical transmission is to increase the external magnesium ion concentration, which has been shown at many synapses to block the release of transmitter. However, we were unable to demonstate this effect on transmission from the hair plate afferents. Bathing the ganglion in a solution containing a high concentration of magnesium (50 mM) for periods up to 45 minutes did not block the reflex activation of motoneuron $D_s$ by the hair plate afferents nor the production of short latency EPSPs by these afferents. Desheathing the ganglion in this high magnesium solution also did not lead to a block of transmission. The inability of high magnesium solutions to prevent the generation of EPSPs does not exclude chemical transmission for it is possible that there is a marked diffusion barrier between the exterior of the ganglion and the synaptic sites within the neuropile. In some other animals chemically mediated synaptic transmission is also known to be unaffected by high magnesium solutions (Zucker, 1972). Burrows (1975) was also unable to completely block the production of EPSPs in locust flight motoneurons with high magnesium but he did observe a significant decrease in EPSP amplitude over a period of 45 minutes. If a similar decrease in amplitude occurred in the present experiments, it would not be detected since it was impossible to record for long periods of time from the same neuron and the amplitudes of the EPSPs were variable form neuron to neuron, and even in the same neuron on maintained penetration.

A common characteristic of chemical synapses but not electrical

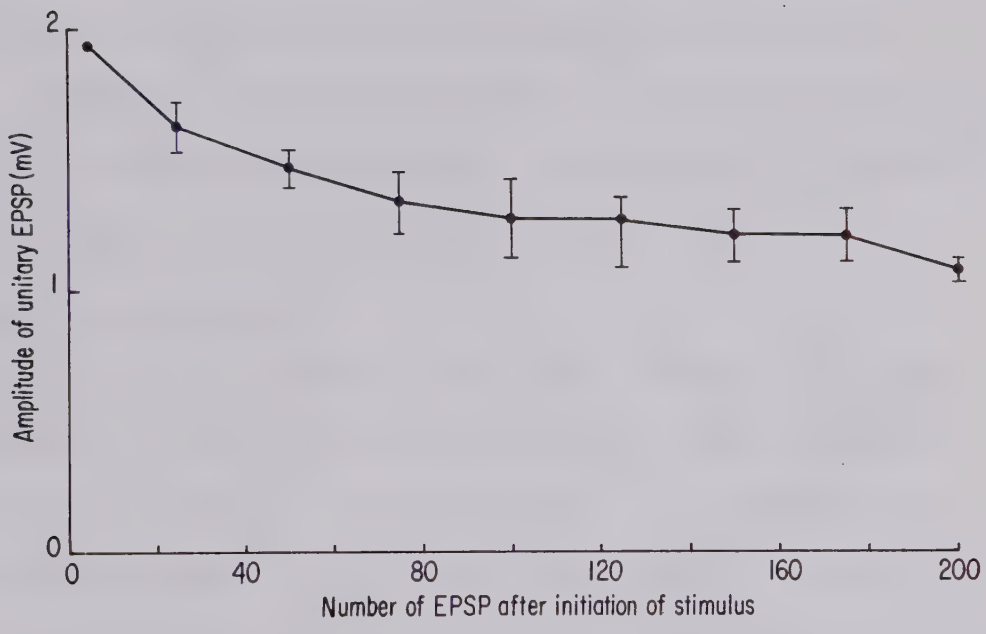


Figure 30. Shows a plot of the time course of the amplitude of the unitary EPSPs evoked by high frequency stimulation (100 Hz) of the hair plate sensillum.

synapses is a facilitation and/or depression of EPSP amplitude following repetitive stimulation (Bennett, 1974). For a wide range of stimulation frequencies I never observed any facilitation in EPSP amplitude but with high frequency stimulation there was a progressive diminution in amplitude. Stimulation at 100 per second for 1 second decreased the amplitude of the EPSPs evoked immediately following the stimulus train to about 75% of the initial value (figure 30).

(iii) *Inhibitory postsynaptic potentials*

In the previous chapter it was shown that the slow flexor motoneurons to the posterior coxal levator muscles were inhibited by stimulating the hair plate. On three occasions I penetrated the neurites of slow motoneurons to these muscles and observed an inhibitory postsynaptic potential in response to electrical stimulation of the hair plate (figure 31). It was not possible to individually identify the flexor motoneurons from which recordings were made, but since the region of penetration contains the main neurites of the motoneurons 5 and 6 (Pearson & Fourtner, 1975) it is probable that on at least one occasion, one of these neurons were penetrated. IPSPs were also recorded from unidentified nonspiking interneurons in regions close to main neurites of the flexor motoneurons (deep within the lateral edge of the ganglion approximately level with nerve 3). Since the amplitudes, durations and latencies of the IPSPs in the motoneurons and the nonspiking neurons were similar, no attempts will be made to distinguish between the neurons in the following description of the characteristics of the IPSPs.

The amplitude of the IPSPs depended upon the number of

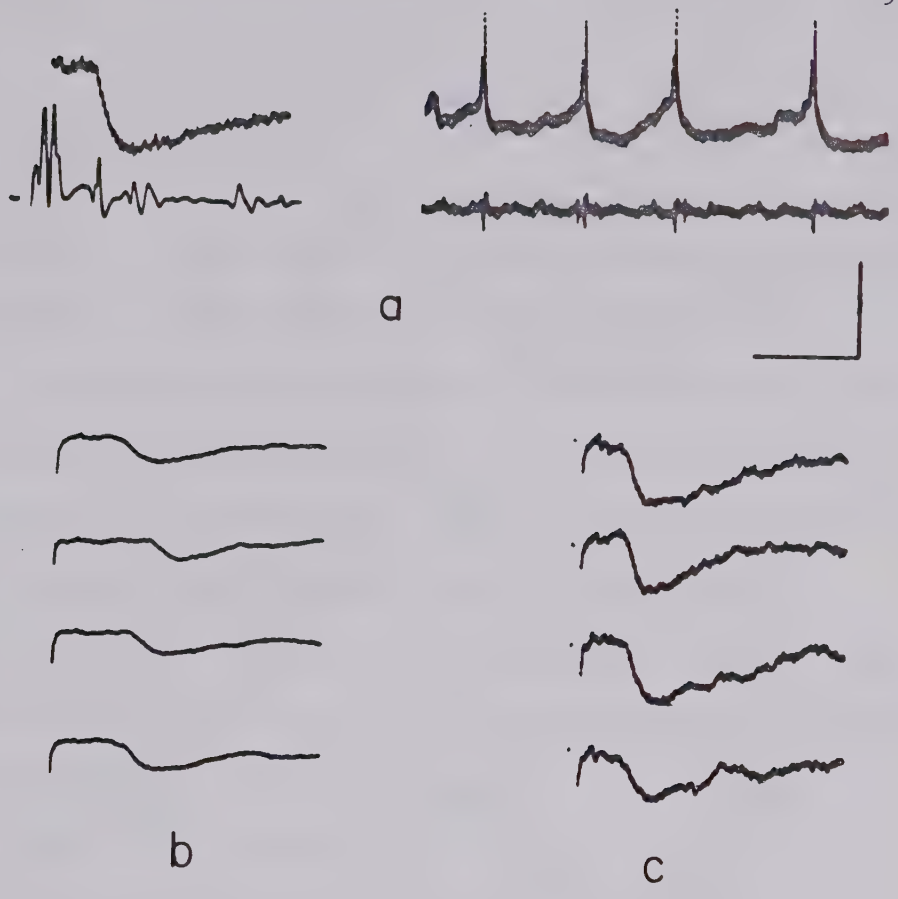


Figure 31 IPSPs recorded from flexor motoneurons (a and b) and a nonspiking neuron (c) evoked by stimulation of the trochanteral hair plate afferents. (a) Left - IPSP in flexor motoneuron, the bottom trace is the nerve 5 record; right - recording in same motoneuron showing 1:1 correspondence between intracellularly recorded spikes (top) and extracellularly recorded junctional potentials from the posterior coxal levator muscle (bottom trace). (b) A series of IPSPs recorded in a flexor motoneuron on repeated stimulation of the hair plate afferents showing variations in latency. (c) A series of IPSPs recorded in a nonspiking neuron on repeated hair plate stimulation showing slmost no variation in latency. Calibrations: amplitude - (a) and (c) 4 mV, (b) 8 mV; time - (a) left, (b) and (c) 8 msec., (a) right 50 msec.

active afferent fibers. Only rarely was an IPSP produced by activation of a single afferent. On most occasions a number of afferents had to be synchronously activated to evoke an IPSP. With strong stimulation of the hair plate the peak amplitude of the IPSPs could be as high as -5 mV. The durations of the IPSPs were within the range of 15 to 30 msec. and thus slightly longer than the duration of the EPSPs.

The latencies of the IPSPs were in the range of 2.5 to 4 msec. and on average about 1.8 msec. longer than the mean latency of the EPSPs occurring in other neurons (Table 1 and figure 27 ). The variation in latency of the IPSPs in any one neuron was usually (7 out of 10) very small for repeated stimulation of the same group of afferents and may be regarded as constant (figure 31c). This variability was not noticeably different from that of the EPSPs described above (figure 26 ). By contrast in one motoneuron and two nonspiking neurons the latency of the IPSPs showed considerable variation with repeated stimulation of the same afferent fibers (figure 31b). The mean values of the variable latency IPSPs were similar to those of the constant latency IPSPs and the range of variation was between 2.5 and 4 msec. Because the neurons in which IPSPs were recorded cannot be identified, it was impossible to determine whether the individual neurons in which the latency of the IPSP was variable differed from those in which the latency was constant. Clearly this is an important point since the pathway for eliciting the constant latency IPSP may differ from the pathway eliciting the variable latency IPSP. It was observed however, that both motoneurons and interneurons could have constant and variable latency IPSPs.

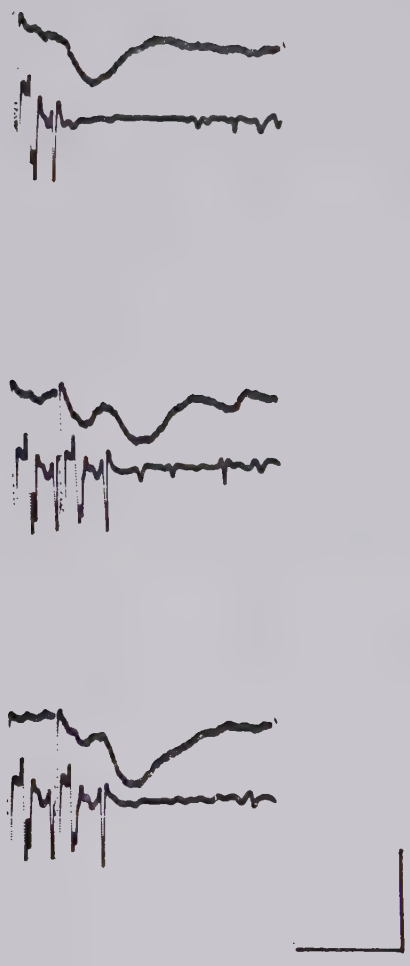

Figure 32. IPSPs recorded in a nonspiking neuron evoked by stimulation of the hair plate afferents. Top traces - intracellular record; Bottom traces - nerve 5 record. Synchronized sensory volley elicited from the hair plate evoke an IPSP in this neuron (top record). Double shocks eliciting two afferent volleys evoke two IPSPs that summed. Discrete IPSPs are evoked even when the sensory volley are arriving at a close interval of 3.5 msec. (bottom record). Calibration: intracellular record, Amplitude - 4 mV; Time - 8 msec.

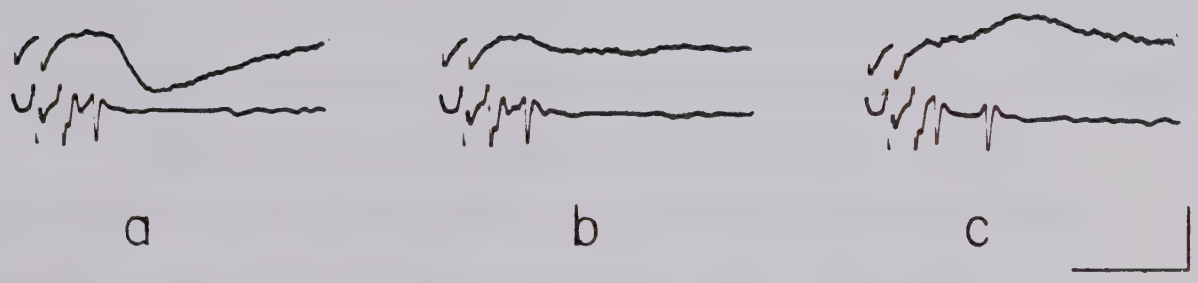


Figure 33. Reversal of IPSP in a nonspiking neuron by the passage of a hyperpolarizing current. To elicit the IPSP the hair plate afferents were stimulated by two pulses separated by 2 msec. No IPSP was elicited by single pulse stimulation. Top traces - intracellular records; bottom traces - nerve 5 records. (a) Current = 0 nA; (b) current - 7.5 nA; (c) current - 14 nA. Calibrations: IPSP amplitude - 4mV; time - 8 msec.

Both constant and variable latency IPSPs could be elicited 1:1 by stimulation of the hair plate afferents at frequencies up to 60 per second. Beyond this frequency fusion and diminution in amplitude of the IPSPs prevented us determining whether they continued to be elicited 1:1. In two pulse stimulation experiments two IPSPs could be elicited with the interpulse interval as short as 3.5 msec. (figure 32 ). Thus the inhibitory synaptic link when activated appeared to be very secure.

Injection of small hyperpolarizing currents diminished the amplitude of the IPSPs while large hyperpolarizing currents reversed the IPSPs (figure 33). The reversal of the IPSPs demonstrates that these are generated by a conductance change with an equilibrium potential more negative than the resting membrane potential. Thus it can be concluded that the IPSPs are produced in the conventional manner by an inhibitory chemical transmitter and not by the removal of a tonic excitatory input to the neurons. No further tests for chemical transmission such as measuring postsynaptic conductance changes, studying the effects of high magnesium and of chloride ion injection were conducted.

(iv) *Postsynaptic response to mechanical stimulation of the trochanteral hair plate*

Step displacements were applied to stimulate the hair plate (see chapter 2 for method). Figure 34 shows a record of the excitatory postsynaptic potentials evoked in the fast extensor motoneuron by activity in the hair plate afferents. Both the onset and release of the displacement excited the hair plate afferents and evoked EPSPs in the motoneuron. Unitary EPSPs were evoked by the afferent impulses

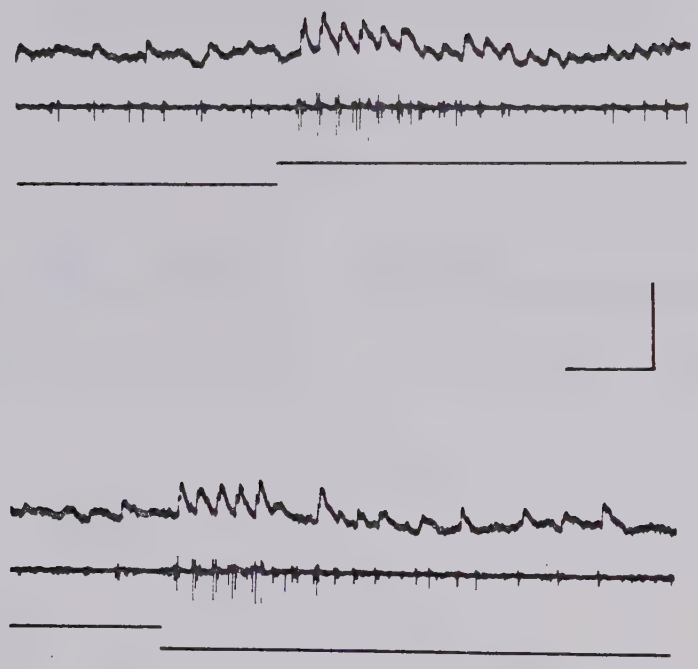

Figure 34. EPSPs recorded from the fast extensor motoneuron evoked by mechanical stimulation of the hairs of the trochanteral hair plate. Top traces - intracellular record; Middle traces - nerve 5 record; Bottom traces - record of the level of mechanical displacement of the hairs; upward deflection signals the onset of displacement, downward deflection signals the release of the displacement. Calibration: intracellular record, Amplitude - 8 mV; Time - 50 msec.

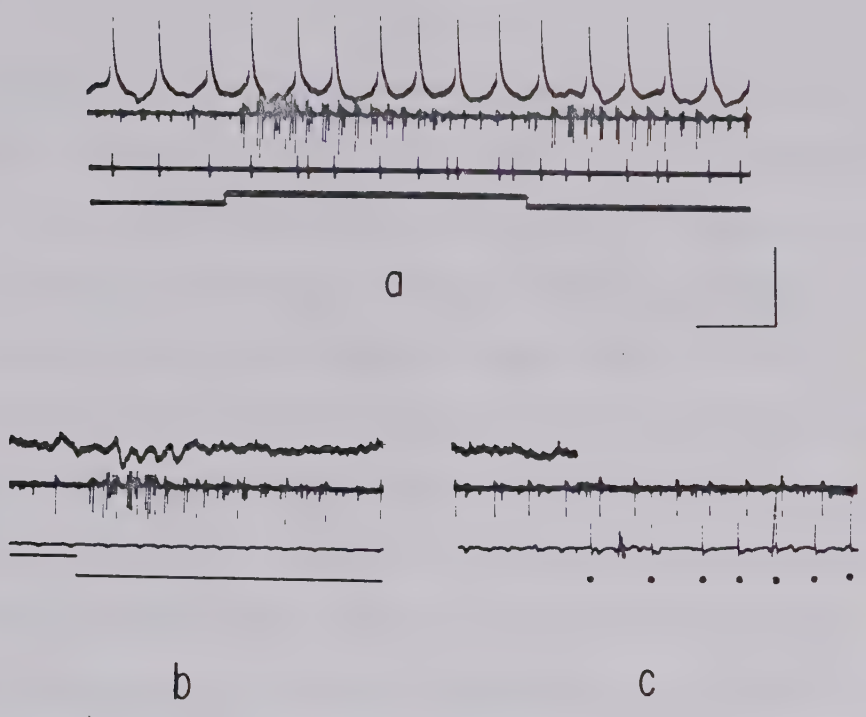


Figure 35. IPSPs recorded from flexor motoneurons evoked by afferent activity from the trochanteral hair plate sensilla. Top traces - intracellular records; second traces (from top) - nerve 5 record; third traces - nerve 6 record monitoring extracellular activity of flexor motoneurons; bottom traces - level of mechanical displacement. Record (a) is obtained from one flexor motoneuron and record (b) & (c) is obtained from another motoneuron. Notice the 1:1 correlation of the intracellular recorded spikes and the extracellularly recorded impulse in (a). The afferent activity evoked during the onset and release of the displacement evoked IPSPs in these motoneurons. In record (c) current is injected into the motoneuron resulting in a break in the intracellular record. Spikes evoked in the motoneuron is recorded from nerve 6. Calibrations: Intracellular record; amplitude - 8 mV; time - 50 msec.

These unitary EPSPs summed when the afferent impulses were arriving at close intervals. Figure 35 shows IPSPs evoked in the flexor motoneurons by the activity in the hair plate afferents. It seemed that individual IPSPs were generated by a number of afferent impulses. Furthermore, activity in the hair plate afferents did not always generated observable IPSPs. Characteristics of EPSPs and IPSPs evoked in nonspiking neurons resembled those recorded in the extensor motoneurons and flexor motoneurons respectively. These observations suggest that the inhibitory synaptic link formed by the hair plate afferents with the flexor motoneurons and nonspiking neurons is not as easily activated as the excitatory synaptic link formed by the hair plate afferents with the extensor motoneurons and nonspiking interneurons.

(v) *Diphasic potentials evoked in nonspiking neurons*

This type of nonspiking neurons were identified not only physiologically from the type of response evoked in them by the activity of the hair plate afferents but also by their location in the ganglion. In four preparations successful recordings were obtained when the microelectrodes were made to penetrate the lateral edge of the ganglion level with the posterior edge of nerve 3. Although it seemed very probable that the same neuron was penetrated in every case, the possibility of the existence of a localized population of these nonspiking neurons could not be excluded. Figure 36a shows the unitary excitatory postsynaptic potential evoked in the nonspiking neuron by an afferent impulse from the hair plate. The synaptic noise recorded in this neuron was usually very high. The unitary EPSPs summed with the synaptic noise. The latency of the onset of the unitary EPSPs measured from the negative

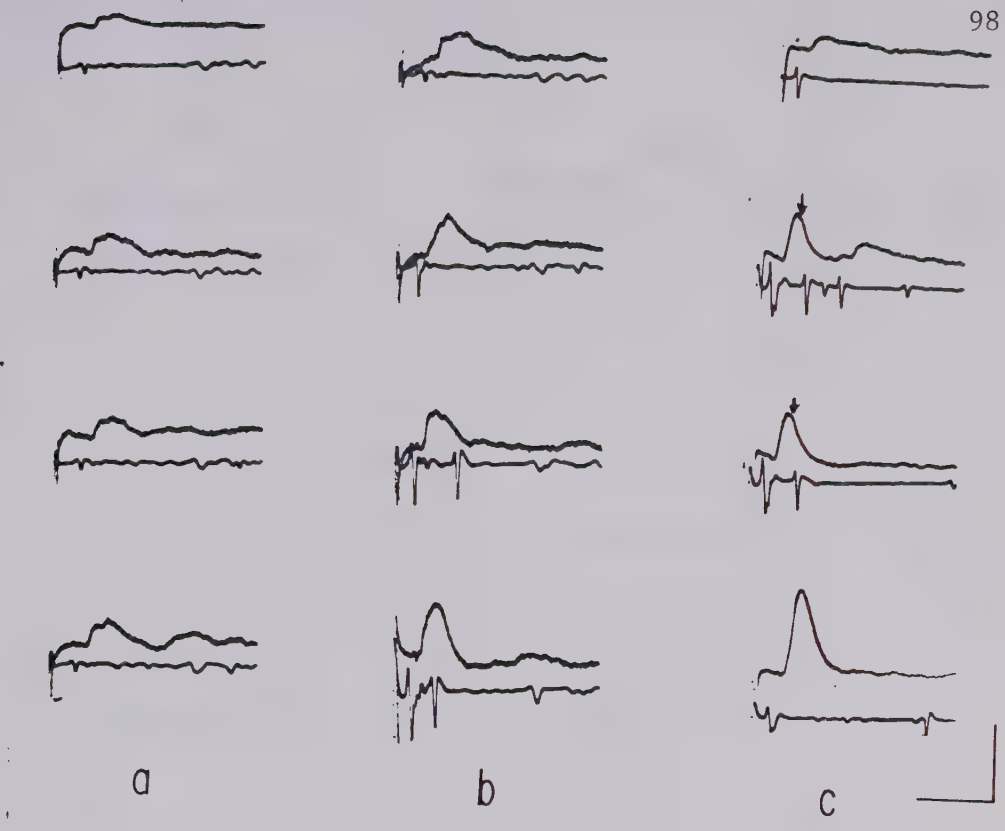


Figure 36. Postsynaptic responses in a nonspiking neuron evoked by electrical stimulation of the hair plate afferents. Top traces - intracellular record; Bottom traces - nerve 5 record. (a) & (b) are recorded from the same neuron. In (a) unitary EPSPs are generated after a constant latency by afferent impulses recorded from nerve 5. The synaptic noise of the neuron is high. The EPSPs summed with the noise. (b) Increasing the stimulus current to the hair sensilla evoked larger sensory volleys. The bottom record shows that with the large sensory volley, a biphasic postsynaptic potential is evoked (depolarizing - hyperpolarizing) in the neuron. (c) Same effect of increasing sensory volley on the postsynaptic response recorded from a nonspiking neuron in another preparation. The two arrows in the second and third records from the top indicate a discontinuity in the recorded postsynaptic potential caused by the onset of the hyperpolarizing phase. Calibration: intracellular records, amplitude - 4 mV; time - 8 msec.

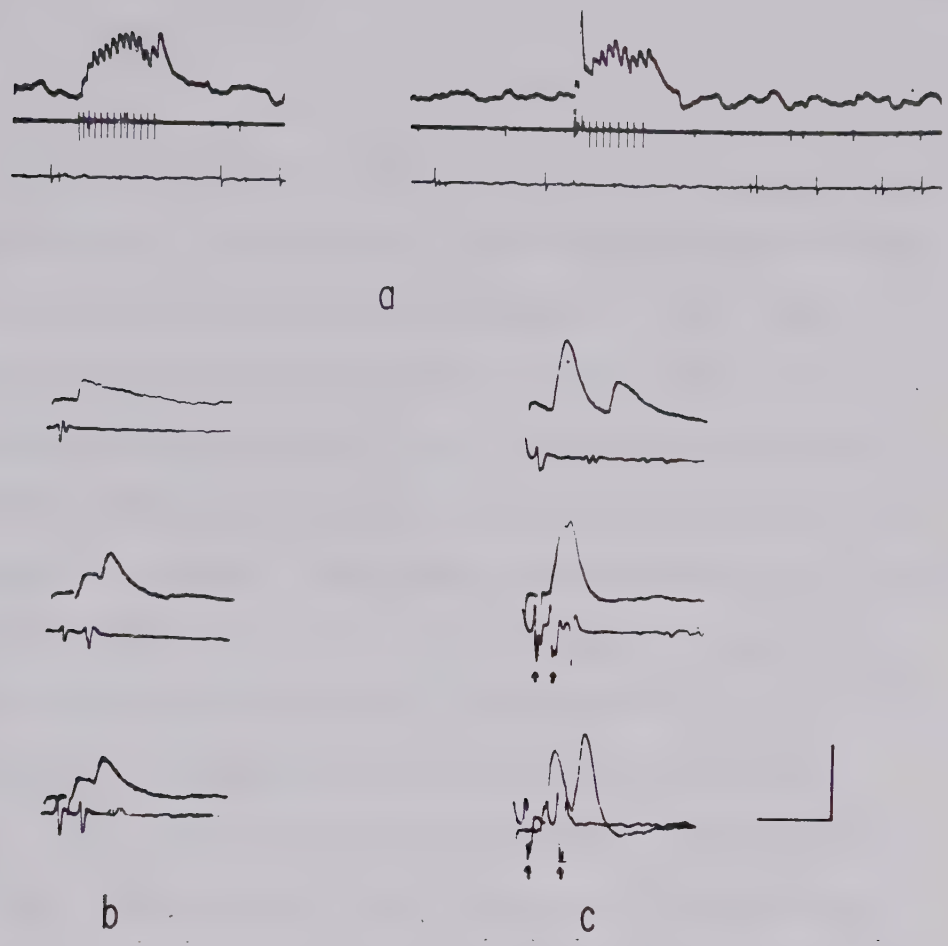


Figure 37. Postsynaptic potentials recorded from a nonspiking neuron evoked by hair plate afferent activity. Top traces - intracellular record; middle traces - nerve 5 record; bottom traces - nerve 6 record. (a) Left record, spontaneous activity in a hair plate afferent and unitary EPSPs evoked in the neuron, right record, electrical stimulation of the hair sensilla and postsynaptic response in the neuron. (b) Unitary EPSPs evoked in the nonspiking neuron summed when they are generated by two closely spaced afferent impulses. (c) Biphasic potentials generated by synchronized sensory volleys elicited from the hair plate. When the two afferent volleys are closely spaced, (second record from top), the second volley does not generate any depolarization. Calibration: intracellular record, amplitude - 4 mV; time - (a) 50 msec., (b) & (c) 8 msec.

peak of the triphasic potential recorded from afferent axon in nerve 5 was extremely constant. As the size of the afferent volley increased by increasing the the stimulus current applied to the hair plate sensilla, the postsynaptic response also increased. When a critical size of the sensory volley was evoked, the shape of the evoked postsynaptic potential changed and consisted of two phases (figure 36b,c). These two phases are an initial depolarizing phase followed by a hyperploarizing phase. Results on double pulse experiments showed that when the second sensory volley arrived at the nonspiking neuron during the onset of the hyperpolarizing phase caused by the first sensory volley, the second sensory volley could not cause any depolarizing effect in the neuron (figure 37c). This suggest that the hyperpolarizing phase was caused by an increase in the membrane conductance of the postsynaptic nonspiking neuron. In some records, the onset of the hyperpolarizing phase was abrupt causing discontinuity in the recorded postsynaptic potential (figure 36c). The latency of the onset of the hyperpolarizing phase measured from the negative peak of the afferent volley recorded from nerve 5 was 2.4 msec. This value of latency is in the range of the latencies measured for IPSPs evoked in other neurons by the hair plate afferents.

## 4.4. DISCUSSION

### (i) *Impulse conduction block in the terminal branches of the hair plate afferent fibers*

Intracellular recordings made in the terminal branches of the hair plate afferents showed that following one impulse the conduction of a second impulse may be blocked in some terminals if the second

impulse occurs within a short interval after the first impulse. On theoretical grounds, it is probable that this block occurs at the branch points. All the records in the experiments were obtained within 30 minutes of the beginning of the experiment and mostly upon first penetration of the ganglion. It can therefore be assumed the blockage of impulse conductance in the sensory terminal branches is not due to deterioration of the preparation since recordings of postsynaptic activities and spontaneous neuronal activities can still be obtained in the preparations for well over an hour. Although the significance of conduction block in the afferent terminal is not yet clear, it is unlikely that this effect could be negligible. The longest minimum-response-interval obtained from the result in one afferent terminal is 6 msec (figure 22), suggesting that some of the afferent impulses arriving at 170 Hz and above will be blocked and will not invade the terminal. In chapter 2 it has been shown that transient responses of well above 170 Hz can be elicited from the hair plate receptors by mechanical stimulation of the hair plate. If the branch points are the regions of conduction block, they will filter out some of the high frequency afferent impulses generated by the hair plate receptors during normal function. Moreover, if the excitability of the branch points can be influenced by changes in factors such as $pCO_2$, extracellular potassium ion concentration and electrical events in adjacent neurons, as suggested by a number of previous studies (Barron, & Matthews, 1939; Grossman, spira, & Parnas, 1973; Krnjevic, 1974; Morris, 1971; Wall, Lettvin, McCulloch & Pitts, 1956), then conduction block in the branch points could be an important

mechanism for regulating sensory input.

The results also demonstrated that for some afferent fibers impulse conduction to their terminals is not blocked by any amount of reduction in excitability during the relative refractory period in their axons and branch points (figures 22, 23, 24 ). For other afferents, blockage of presynaptic impulses does occur but the minimum-response-interval is not the same for the terminal region of different afferents. This indicates that for different parts of the terminal branches, the reduction in safety factor for impulse conduction is not the same. The difference in reduction of safety factor is probably due to a difference in the geometry of the various afferent fibers. Thus the branching pattern of afferent terminals could be of importance in the transmission of sensory impulses throughout the ganglion.

(ii) *Excitatory postsynaptic potentials*

In the previous chapter I described an excitatory reflex pathway in the cockroach metathoracic leg from the trochanteral hair plate afferents to the slow motoneuron producing extension movements of the femur in a walking animal (motoneuron $D_s$). Some data in that study indicated that transmission in this reflex pathway could be monosynaptic. One of the main conclusions of the present investigation is that the trochanteral hair plate afferents do monosynaptically connect to motoneuron $D_s$, as well as to at least one other extensor motoneuron and to a number of unidentified nonspiking neuron, and transmission at these junctions is mediated by a chemical transmitter.

The conclusion that the EPSPs are elicited monosynaptically

follows from the observation of a short constant delay ( 0.4 msec.) between the peak of the afferent terminal spike and the beginning of the EPSPs. This latency is too short for the EPSPs to be generated via a disynaptic pathway with chemical transmissions in the synapse, and too long for the EPSPs to be generated monosynaptically via an electrical junction. Thus the EPSPs must be produced either monosynaptically via a chemically transmitting junction, or disynaptically with either the first or both junctions being electrical. Since no postsynaptic potentials were recorded with latencies as short as the afferent terminal spikes (figure 27) the possibility of the hair plate afferents making electrical synaptic connections on any neurons within the ganglion must be considered unlikely. However, it is conceivable that recordings were not made from the intercalated neurons either because of their small size or their location in regions of the ganglion other than those penetrated. Despite this reservation the simplest explanation of the experimental data is that the EPSPs are elicited monosynaptically via a chemically transmitting synapse. In addition to a short synaptic delay, further evidence for chemical transmission is the increase in amplitude of the EPSPs on the passage of a hyperpolarizing current (figure 29), and the diminution of the EPSP amplitude following high frequency stimulation of the hair plate afferents. The failure of high concentrations of magnesium ions to abolish the EPSPs does not exclude chemical synaptic transmission. It is possible that there is a barrier which prevents the diffusion of magnesium ions to presynaptic sites within the neuropile. Chemical synaptic transmission at some junctions in the crayfish abdominal ganglia are also resistant to elevated concentrations of magnesium ions

(Zucker, 1972), as is chemical transmission at some junctions in the mollusc Aplysia (Tauc et al., 1965).

(iii) *Inhibitory postsynaptic potentials*

The main question to be considered in this part of the discussion is whether or not the inhibitory postsynaptic potentials recorded in flexor motoneurons and some unidentified nonspiking interneurons are monosynaptically elicited by spikes in the hair plate afferents. The importance of this question is that at present there is no convincing example of inhibitory afferents in any arthropod system. The IPSPs occurring in elevator motoneurons of the locust flight system in response to activity in wing stretch receptor afferents may be monosynaptically elicited (Burrows, 1975), but the possibility that this inhibitory pathway is di- or polysynaptic has not been excluded.

The long latency of the IPSPs following the spike in the afferent terminals (figure 27) is the first indication that the connection is not monosynaptic. The value of this latency, 1.5 to 4 msec., is significantly greater than the value for synaptic delay conventionally taken to be about 0.5 msec. However, it may be that the synaptic delay at central inhibitory synapses in the cockroach is larger than the delay at synapses in other animals. Long latency monosynaptic excitatory connections have been described in the leech (Nichols and Purves, 1970) while the latency at some inhibitory synapses in molluscs can be as high as 3 msec. (Kandel et al., 1967). Another possible reason for the long latency of the IPSPs is that conduction along the inhibitory terminal branches may be slower than that along branches giving excitation. If this was so then the inhibitory

branches would presumably be very small and not allow microelectrode penetration. This would then explain the failure to record the long latency afferent terminal spikes expected in the inhibitory afferent terminals. Since long latency extracellular field potentials from the afferent terminals were never recorded, it seems unlikely that the conduction velocity in the terminals leading to the IPSPs is slow.

More compelling reasons for believing that the IPSPs are not monosynaptically elicited are that usually more than one afferent fiber is required to produce an IPSP and that there is a variability in IPSP latencies in some neurons. Neither of these two effects can be readily explained if the connection is monosynaptic. It is conceivable that the IPSPs elicited by a spike in a single afferent are too small to be detected above the noise, while variability in the latency may be due to unusually high variability in the stochastic processes underlying chemical transmission at the inhibitory synapses. However, both these effects, as well as the long latency of the IPSPs, are far more readily explained by assuming that the IPSPs are elicited via a disynaptic pathway involving a single intercalated inhibitory interneuron. The need for a synchronous afferent volley to elicit the IPSPs is then explained by the need for summation of EPSPs in the inhibitory interneuron either to produce an action potential or to depolarize the interneuron to a level where inhibitory transmitter is released in a graded manner. The variability in latency would then be due to variation in the time for the membrane potential to reach this threshold level as a result of spontaneous fluctuations in membrane potential which are known to occur in some interneurons

(Pearson & Fourtner, 1975). Thus it is more probable that the IPSPs are produced disynaptically. It remains to consider whether action potentials in the inhibitory interneuron are required for transmitter release.

The observation that the variability in the latency of the IPSPs is usually very small (figure 31c ) immediately suggests that graded depolarization in the inhibitory interneuron is responsible for the production of the IPSPs. When recordings were made from neurons in which spikes were elicited by stimulation of the hair plate afferents the variability in the latency of the spikes was always larger than the variation in latency of the IPSPs (for example see figure 13 and compare with figure 31b, c ). Thus the action potentials in none of the spiking neurons recorded could have produced the IPSPs. of course it is conceivable that the IPSPs were elicited by action potentials in interneurons not penetrated in the experiment. However, since it is known that there are many nonspiking interneurons in the cockroach ganglion and no spiking neurons have yet been found to influence activity in flexor motoneurons (Pearson & Fourtner, 1975), the simplest explanation for the data is that the IPSPs are produced by the graded depolarizations in nonspiking interneurons. Some of the nonspiking interneurons found to be monosynaptically excited by hair plate afferents could obviously be the interneurons in the inhibitory pathways to the flexor motoneurons and some nonspiking interneurons. At least three nonspiking interneurons can inhibit activity in the flexor motoneurons (Pearson & Fourtner, 1975), but there is no evidence to clearly determine whether any of these interneurons are monosynapti-

cally excited by the hair plate afferents.

(iv) *Biphasic potentials evoked in nonspiking neurons*

Sychronous activity in a number of hair plate afferents evoked an initial depolarizing and subsequent hyperpolarizing response in some nonspiking neurons. Probably the initial depolarization evoked in the nonspiking neuron by the hair plate afferent activity raised the membrane potential to such a level that an outward current is activated, causing the hyperpolarizing phase. However the abrupt onset of the hyperpolarizing phasesuggest that the hyperpolarizing phase is not coupled with the depolarizing phase. The latency of the onset of the hyperpolarizing (2.4 msec.) suggest that it is evoked by the activity in the hair plate afferents via an intercalating inhibitory neuron. An observation which tends to support this postulation is that a number of afferents have to be activated before the hyperpolarizing phase is observed (figure 36). It can thus be concluded that the hair plate afferents establish monosynaptic excitatory and disynaptic inhibitory connections with these nonspiking neurons.

(v) *Comparison with other systems*

The organization and properties of the reflex pathways from the hair plate afferents to the femur flexor and extensor motoneurons described in this paper are similar to those in the locust flight system described by Burrows (1975). In both systems the primary afferent fibers give monosynaptic, chemically mediated, excitation of one group of motoneurons and inhibition of the antagonistic group of motoneurons. The latency of the IPSPs is from 1.5 to 3 msec. later than the EPSPs in both systems, it is therefore

probable that the IPSPs are elicited via a nonspiking inhibitory interneuron. Furthermore, in both systems activity in the group of motoneurons receiving the inhibition causes movements which excite the sensory afferents. Thus the reflex pathways are organized so as to terminate the movements in one direction and promote the initiation of the opposite movement. The possible functional advantages of organizing the reflex pathways in this manner have been discussed in chapter 3.

Monosynaptic reflex pathways onto motoneurons are well known in vertebrates but have only recently been identified in invertebrates. Burrows (1975) has clearly demonstrated a monosynaptic excitatory reflex pathway in the locust from wing stretch receptor afferents to wing depressor motoneurons, and in the crab Sandeman (1969) has described a monosynaptic excitatory connection from head hair receptors to motoneurons giving eye withdrawal movements. The present finding of the monosynaptic connection from trochanteral hair receptor afferents to femur extensor motoneurons is thus a third example of a monosynaptic reflex pathway in arthropods. The existence of these pathways together with the finding of monosynaptic reflex pathways in the leech (Nichols and Purves, 1970) and the mollusc *Aplysia* (Castellucci *et al.*, 1970) clearly indicates that monosynaptic excitatory connections of sensory afferents onto motoneurons may be widespread in invertebrates.

A type of biphasic postsynaptic response is described in *Aphysia californica* (Wachtel and Kandel, 1967, 1971). It consists of a coupling of excitatory and inhibitory actions observed in neuron L7 when neuron L10 is made to fire by direct intracellular stimulation.

Direct Ach application by iontophoresis to the somatic membrane of neuron L7 also causes a biphasic response composed of an early depolarization and a slower hyperholarization. Evidently both phases of the response are evoked by the same transmitter (Ach). In the present experiment, the possibility that the biphasic response obtained in the nonspiking neuron is evoked by a dual action of the presynaptically released transmitter cannot be excluded.

# CHAPTER 5

## FUTURE WORK

The present study is part of an investigation to examine how specific receptors interact with the centrally programmed motor output to produce the motor pattern underlying walking. The results of the study show that the inputs from the trochanteral hair plate play a significant role in the control of walking. The extent to which a number of other proprioceptors are involved in the control of walking awaits future investigations. In the following section, future work pertinent to the study of reflex control in walking and specific experiments designed to further investigate the function of the hair plate will be discussed under the appropriate headings.

### (i) *Response of the trochanteral hair plate sensilla to femur flexion*

In the present study, the properties of the sensilla of the hair plate were studied by an analysis of their response to displacement by an insect pin. The response of the hair sensilla to the flexion of the femur was not investigated. During femur flexion, a large number of proprioceptors in addition to the hair plate are excited. (e.g. the campaniform sensilla and the multipolar receptor). The afferents of all these proprioceptors are contained in nerve 5. Hence electrodes placed on nerve 5 will record the summed discharges from the proprioceptors during femur flexion and it will not be possible to study the hair plate response from such a record. In chapter 2, it was suggested that the hair plate sensilla are activated only when the femur is in a relatively flexed position. This suggestion is based on the observation

that the hair plate sensilla are not physically displaced by the intersegmental joint membrane until the femur is in a relatively flexed position. In order to fully understand the function of the hair plate in a walking animal, it is essential to obtain detailed information on how the hair plate sensilla is activated by the imposed or spontaneous flexion movements of the femur. Since the extracellular activity of single afferents from the hair plate can be easily recorded with microelectrodes placed in the ganglion and such recordings are usually quite stable (see chapter 4), a simple approach to investigate the response of the hair sensilla to femur flexion is to record the afferent activity extracellularly in the ganglion. The hair plate afferent will first be activated by electrical stimulation of the sensillum. Once an extracellular recording of the afferent activity in the ganglion is obtained, the response of the hair plate sensillum to either imposed or spontaneous flexion of the femur can be recorded. Results from these studies will provide information on (1) the position during femur flexion at which the hair sensillum is activated, (2) the velocity or frequency of femur flexion required for the activation of different type I sensilla and (3) the importance of type II sensilla in the control of leg posture. Furthermore extracellular recordings from the hair plate afferent terminal branches will permit the study of impulse conduction in these terminal branches when high frequency impulses are elicited from the hair plate sensillum during the spontaneous flexion movements of the femur.

(ii) *The study of the effect of hair plate afferent activity onto identified interneurons*

A number of nonspiking interneurons in the cockroach walking

system has been identified and labelled (Pearson & Fourtner, 1975). Since the activity in at least three of the identified nonspiking interneurons can inhibit the spiking activity in the flexor motoneurons, one of the questions that arised from the present investigation is whether the inhibitory effect of the hair plate afferents onto the flexor motoneurons is mediated via these interneurons. Nonspiking interneurons can be identified physiologically by recording their activity intracellularly and examining their influence on the motoneurons. They can also be identified anatomically and physiologically by the application of dye filled microelectrodes. The influence, if any, of the activity in the hair plate afferents onto these identified interneurons will be examined and the results may provide further information on the characteristics of the inhibitory pathway from the hair plate to the femur flexor motoneurons.

The system of interneurons producing the flexor bursts (central oscillator) in the cockroach has been investigated and one of the interneurons in this system has been identified and labelled as interneuron I (Pearson & Fourtner, 1975). Results obtained by extracellular studies in the present investigation showed that the afferent activity from the trochanteral hair plate directly influence the system of interneurons producing the flexor burst (figure 16 ). Experiments will be performed to investigate the effect of the afferent activity from the hair plate onto interneuron I. Results of these experiments will be of general interest because at the present time there is no direct evidence, from intracellular recordings, showing that primary afferents terminate on neurons of any rhythm generating system.

(iii) *The study of proprioceptive feedback in the cockroach walking system*

The relatively simple and accessible motor system producing the flexion and extension movements of the femur during walking in the cockroach (see method section in chapter 3 for a description of this motor system) provides an excellent preparation for the study of the function of different proprioceptors in the control of walking. The anatomy of a large number of sensory organs in the mesothoracic leg of the cockroach is described in much detail by Nijenhuis and Dresden (1952, 1954). Preliminary studies have been conducted to investigate the reflex connections of the trochanteral campaniform sensilla and the femoral chordotonal organ with leg motoneurons. The results of these studies will be briefly described here along with the possible future direction of investigation.

Previous studies indicated that input from the trochanteral campaniform sensilla excites motoneuron $D_s$ and inhibits the flexor burst generator (Pearson, 1972). The importance of these reflex connection in load compensation and intersegmental coordination in a walking animal has also been discussed (Pearson, 1972; Pearson & Iles, 1973). Three groups of the trochanteral campaniform sensilla are located on the ventral surface of the trochanter and they are extremely accessible. In a priliminary study, individual sensillum was excited by applying pressure on the cap of the sensillum with an insect pin (fig.38) (punctate stimulus). Figure 39 shows a record from nerve 5 of the response of a sensillum to the punctate stimulus and the activation of motoneuron $D_s$ by the sensory input. Afferent from this sensillum therefore established excitatory connection with motoneuron $D_s$. Intracellular studies will be performed to examine the synaptic event evoked in motoneuron $D_s$ and other central neurons by the afferent impulses

Figure 38. Response of a campaniform sensillum to punctate stimulus. The response is recorded from the cut distal end of nerve 5. The first arrow indicate the start of the stimulus, the second arrow indicate a slight increase in the stimulus pressure.

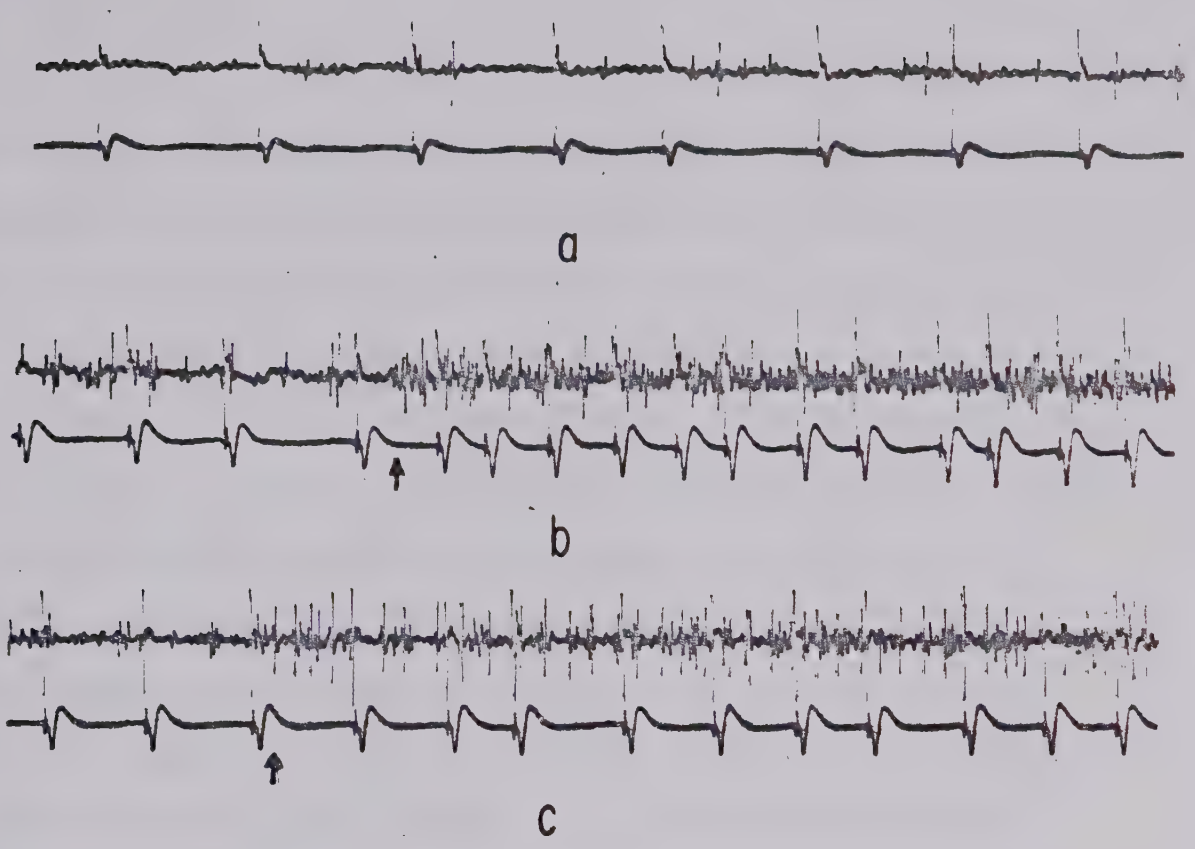


Figure 39. Punctate stimulus activate single sensillum of the trochanteral campaniform sensilla. Top traces - *en passant* nerve 5 record; Bottom traces - record from muscle 177D. (a) No stimulus is applied. Activity of motoneuron $D_s$ recorded from nerve 5 is identified by 1:1 correlation with activity recorded from muscle 177D. (b) Punctate stimulus applied to sensillum evoked afferent activity which is recorded from nerve 5. This afferent activity causes an increase in the activity of motoneuron $D_s$. (c) Punctate stimulus applied to another sensillum generating larger extracellularly recorded impulses also reflexly activates motoneuron $D_s$. Arrows in (b) & (c) signal onset of stimulus.

from the campaniform sensilla. Detailed investigation on the synaptic mechanisms underlying reflex connections may provide information on the function of these connections in the control of walking.

Delcomyn (1971) showed that motoneuron $D_s$ was excited by imposed extension and flexion movements of the tibia and suggested that the reflex acts to augment the thrust of a leg during walking. Although it is not clear which specific proprioceptors were activated when the tibia was extended and flexed, the femoral chordotonal organ must be considered as a possible candidate. The receptor tendon of the femoral chordotonal organ can be easily exposed and cut by opening a small hole on the ventral surface of the femur near the femoral-tibial joint. Preliminary experiments showed that the activity of motoneuron $D_s$ recorded by electrodes placed in the extensor muscle increased during the release of a stretch applied to the receptor tendon of the chordotonal organ. Figure 40 shows a record of excitatory potentials evoked in a fast extensor motoneuron by activity in the afferents of the chordotonal organ. Both the stretching and the releasing of the receptor tendon elicited phasic afferent activity which was recorded from nerve 5. The afferent activity in turn evoked excitatory postsynaptic responses in the motoneuron. The afferent activity from the chordotonal organ also influence (excite or inhibit) a large number of unidentified spiking and nonspiking central neurons(fig.41). In order to study the characteristics of the pathways connecting the femoral chordotonal organ to various central neurons, the afferent nerve will be stimulated electrically to evoke a synchronous sensory volley. The latency between the onset of the postsynaptic events

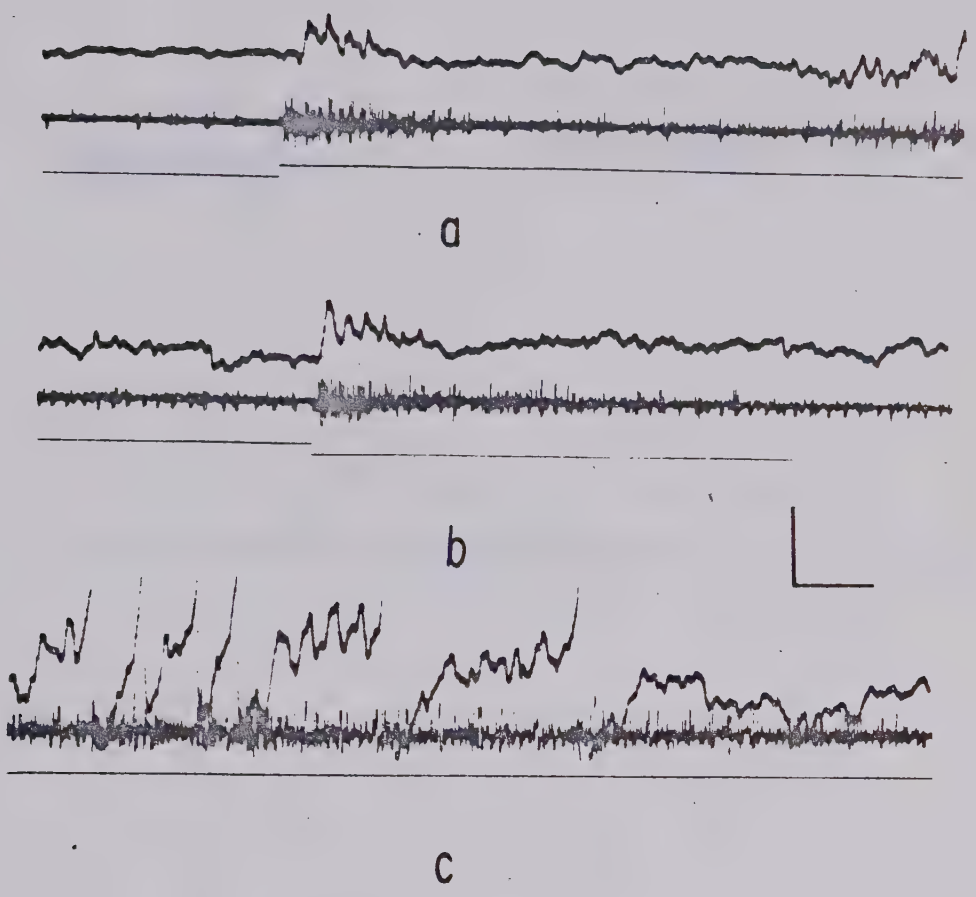


Figure 40. EPSPs in fast extensor motoneuron of the femur evoked by the activity in the afferents of the femoral chordotonal organ. Top traces - intracellular record; Middle traces - nerve 5 record; Bottom traces - stimulus level. An upper deflection signals the stretching of the tendon of the chordontonal organ and downward deflection signals the release. (c) shows the spontaneous firing of the motoneuron.

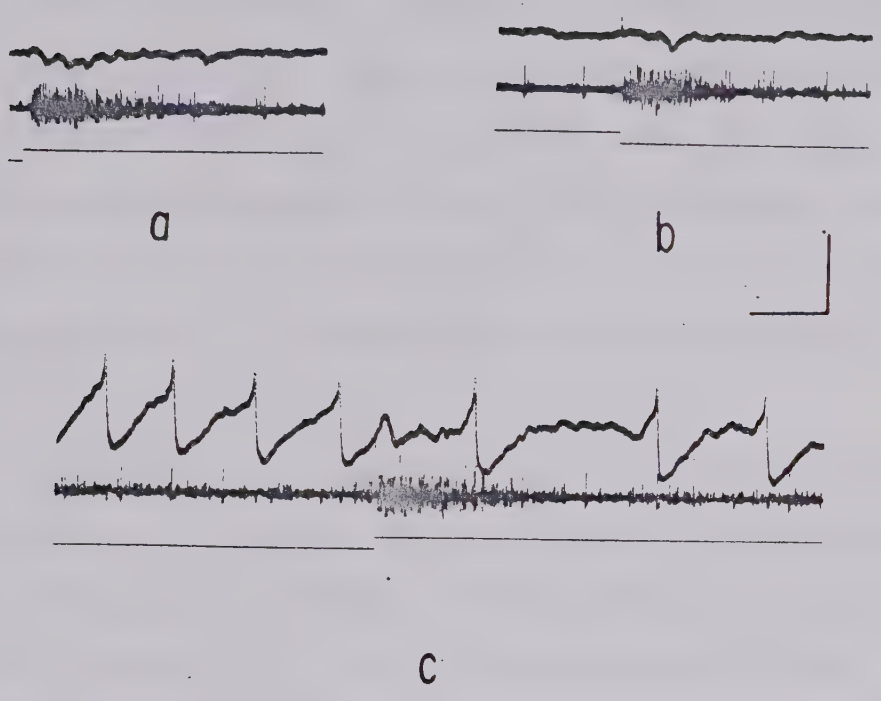


Figure 41. IPSPs in a non-identified neuron evoked by activity in the afferents of the femoral chordotonal organ. Top traces - intracellular record; Middle traces - nerve 5 record; Bottom traces - stimulus level. An upward deflection signals a stretching of the receptor tendon of the femoral chordotonal organ. In (c) the spontaneous firing of the neuron is suppressed by the input from the chordotonal organ.

evoked in different central neurons and the recording of the afferent volley on nerve 5 will be measured. The number of synapses involved in the various sensory to central neuron connections can then be deduced. furthermore, recordings will be made in muscles of a walking animal before and after cutting the receptor tendon of the chordotonal organ. A comparison of the records obtained in the operated and intact animal should provide information on the normal function of the receptor during walking.

Preparations will also be developed to investigate other proprioceptors with regards to their influence on the motor system producing femur extensions and flexions. Results obtained in the study of proprioceptive function in the control of walking are of general interest because they are related to the general problem of obtaining an understanding of the role played by proprioceptors in the control of behaviors generated by rhythmic motor activity.

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